PREVALENCE AND DIAGNOSIS OF ACUTE BACTERIAL MENINGITIS IN A TERTIARY CARE CENTRE

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BONAFIDE CERTIFICATE

This is to certify that the dissertation entitled **"PREVALENCE AND DIAGNOSIS OF ACUTE BACTERIAL MENINGITIS IN A TERTIARY CARE CENTRE"** submitted by **Dr.N.ANURADHA** to the Tamil Nadu Dr. M.G.R. Medical University, Chennai in partial fulfillment of the requirement for the award of M.D degree Branch–IV (Microbiology) is a bonafide research work carried out by her under direct supervision & guidance.

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DECLARATION

I, **Dr. N.ANURADHA** declare that, I carried out this work on, **"PREVALENCE AND DIAGNOSIS OF ACUTE BACTERIAL MENINGITIS IN A TERTIARY CARE CENTRE"** at the Institute of Microbiology, Madurai Medical College. I also declare that this bonafide work or a part of this work was not submitted by me or any others for any award, degree or diploma to any other University, Board, either in India or abroad.

This is submitted to The Tamilnadu Dr. M. G. R. Medical University, Chennai in partial fulfillment of the rules and regulations for the M.D. Degree examination in Microbiology.

Place : Madurai **Dr. N.ANURADHA**

Date :

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CONTENTS

INTRODUCTION

DEFINITION

Bacterial meningitis is an acute purulent infection within the subarachnoid space or throughout the leptomeninges. It is associated with the CNS inflammatory reaction that may result in decreased consciousness, seizures, raised intracranial pressure (ICP) and stroke. The meninges, the subarachnoid space, and the brain parenchyma are all frequently involved in the inflammatory reaction.

EPIDEMIOLOGY

Bacterial meningitis, an infection of the membranes (meninges) and cerebrospinal fluid (CSF) surrounding the brain and spinal cord, is a major cause of death and disability worldwide. Beyond the perinatal period, three organisms transmitted from person to person through the exchange of respiratory secretions are responsible for most cases of bacterial meningitis: Neisseria meningitidis, Haemophilus influenzae and Streptococcus pneumoniae. The etiology of bacterial meningitis varies with age and region of the world. Worldwide, without epidemics one million cases of bacterial meningitis are estimated to occur and 200,000 of these die annually. Case-fatality rates vary with age at the time of illness and the species of bacterium causing infection, but typically range from 3 to 19% in developed countries. Higher case-fatality rates (37-60%) have been reported in developing countries. Up to 54% of survivors are left with disability due to bacterial meningitis, including deafness, mental retardation, and neurological sequelae.

The epidemiology of bacterial meningitis has changed significantly in recent years, reflecting a dramatic decline in the incidence of meningitis due to Haemophilus influenzae, and a smaller decline in that due to Neisseria meningitidis, following the introduction and increasingly widespread use of vaccines for both these organisms.

Currently, the organisms most commonly responsible for community acquired bacterial meningitis are Streptococcus pneumoniae (50%), N. meningitidis (25%), Group B streptococci (15%) and Listeria monocytogenes (10%). H. influenzae now accounts for <10% of cases of bacterial meningitis in most series. Many other microbes and organisms, including Mycobacterium tuberculosis, bacteria, fungi, protozoa and worms, may also cause meningitis or meningeal inflammation. In economically advanced countries, the mortality of bacterial meningitis is less than 10 percent but it may be 30 percent or more in developing countries (Greenwood 1987; Bryan et al. 1990; Bijlmer 1991).

The epidemiology is changing swiftly following the development of conjugated polysaccharide vaccines for invasive Hib disease, for Meningococcal meningitis caused by serogroups A, C, Y, and W-135 and for Pneumococcal meningitis caused by a range of common serotypes. Bacterial meningitis carries a high mortality and morbidity if untreated or treated late. The success of parenteral antibiotic therapy depends on early diagnosis.

BACTERIAL PROFILE OF MENINGITIS

S. pneumoniae is the most common cause of meningitis in adults >20 years of age, accounting for nearly half the reported cases (1.1 per 100,000 persons per year). S. pneumoniae is acquired through the respiratory route. There are a number of predisposing conditions that increase the risk of Pneumococcal meningitis, the most important of which is Pneumococcal pneumonia. Additional risk factors include coexisting acute or chronic Pneumococcal sinusitis or otitis media, alcoholism, diabetes, splenectomy, hypogammaglobulinemia, complement deficiency, and head trauma with basilar skull fracture and CSF rhinorrhea. Mortality remains ~20% despite antibiotic therapy.

N. meningitidis accounts for 25% of all cases of bacterial meningitis (0.6 cases per 100,000 persons per year) and for up to 60% of cases in children and young adults between the ages of 2 and 20. N. meningitidis is classified into serogroups based on the immunological reactivity of the capsular polysaccharide. Although 12 serogroups have been identified, the three serogroups A, B and C account for over 90% of meningococcal disease. Meningococcal disease differs from other leading causes of bacterial meningitis because of its potential to cause large-scale epidemics. A region of sub-Saharan Africa extending from Ethiopia in the East to The Gambia in the West and containing fifteen countries and over 260 million people is known as the “meningitis belt” because of its high endemic rate of disease with superimposed, periodic, large epidemics caused by serogroup A, and to a lesser extent, serogroup C.

During epidemics, children and young adults are most commonly affected, with attack rates as high as 1,000/100,000 population, or 100 times the rate of sporadic disease. The highest rates of endemic or sporadic disease occur in children less than 2 years of age. In developed countries, endemic disease is generally caused by serogroups B and C. Epidemics in developed countries are typically caused by serogroup C although epidemics due to serogroup B have also occurred in Brazil, Chile, Cuba, Norway and more recently in New Zealand.

The presence of petechial or purpuric skin lesions can provide an important clue to the diagnosis of meningococcal infection. In some patients the disease is fulminant, progressing to death within hours of symptom onset. Infection may be initiated by nasopharyngeal colonization, which can result in either an asymptomatic carrier state or invasive meningococcal disease. The risk of invasive disease following nasopharyngeal colonization depends on both bacterial virulence factors and host immune defense mechanisms, including the host's capacity to produce antimeningococcal antibodies and to lyse meningococci by both classic and alternative complement pathways. Individuals with deficiencies of any of the complement components, including properdin, are highly susceptible to meningococcal infections.

H. influenzae causes meningitis in unvaccinated children and adults. Meningitis caused by H. influenzae occurs mostly in children under the age of 5 years and most cases are caused by organisms with the type b polysaccharide capsule (H. influenzae type b, Hib).The frequency of H. influenzae type b

meningitis in children has declined dramatically since the introduction of the Hib conjugate vaccine, although rare cases of Hib meningitis in vaccinated children have been reported. While most children are colonized with a species of H. influenzae, only 2-15% harbour Hib. H. influenzae is acquired through the respiratory route. An essential virulence factor which plays a major role in determining the invasive potential of an organism is the polysaccharide capsule of Hib. Meningitis is the most severe form of Hib disease. In most countries, however more cases and deaths are due to pneumonia than to meningitis.

Escherichia coli K1 are an increasingly common cause of meningitis in individuals with chronic and debilitating diseases such as diabetes, cirrhosis, or alcoholism and in those with chronic urinary tract infections. Gram-negative meningitis can also complicate neurosurgical procedures, particularly craniotomy.

Group B streptococcus or S.agalactiae was previously responsible for meningitis predominantly in neonates, but it has been reported with increasing frequency in individuals >50 years of age, particularly those with underlying diseases.

Neonatal bacterial meningitis (bacterial meningitis in the first month of life) is rare but serious, with a mortality up to 30–40 percent and permanent sequelae in up to 30 percent of survivors. The causative organisms are different from those of bacterial meningitis at other ages, since most bacterial meningitis in this age group is due to organisms derived from ascending infection in utero or from the birth canal during delivery (de Louvois 1994). Occasionally outbreaks occur in hospital

nurseries. Neonatal bacterial meningitis is almost always preceded by bacteremia. In UK, the principal causative organisms are Gram negative enteric bacilli and Group B streptococci. The latter can cause early onset or late onset disease as long as 3–4 months after birth. Other bacteria, including Pneumococci, Listeria monocytogenes, Meningococci, other Streptococci, and Staphylococcus aureus, can also cause neonatal meningitis.

L. monocytogenes has become an increasingly important cause of meningitis in neonates (<1 month of age), pregnant women, individuals >60 years, and immunocompromised individuals of all ages. Infection is acquired by ingesting foods contaminated by Listeria. Foodborne human Listerial infection has been reported from contaminated milk, soft cheeses, and several types of "ready-to-eat" foods, including delicatessen meat and uncooked hotdogs.

Staphylococcus aureus and Coagulase Negative Staphylococci are important causes of meningitis that occurs following invasive neurosurgical procedures, particularly shunting procedures for hydrocephalus, or as a complication of the use of subcutaneous Ommaya reservoirs for administration of intrathecal chemotherapy.

PATHOPHYSIOLOGY

The most common bacteria that cause meningitis, S. pneumoniae and N. meningitidis, initially colonize the nasopharynx by attaching to nasopharyngeal epithelial cells. Bacteria are transported across epithelial cells in membrane bound vacuoles to the intravascular space or invade the intravascular space by creating

separations in the apical tight junctions of columnar epithelial cells. Once in the bloodstream, bacteria are able to avoid phagocytosis by neutrophils and classic complement–mediated bactericidal activity because of the presence of a polysaccharide capsule. Bloodborne bacteria can reach the intraventricular choroid plexus, directly infect choroid plexus epithelial cells, and gain access to the CSF. Some bacteria such as S. pneumoniae can adhere to cerebral capillary endothelial cells and subsequently migrate through or between these cells to reach the CSF. Bacteria are able to multiply rapidly within CSF because of the absence of effective host immune defenses.

Normal CSF contains few white blood cells (WBCs) and relatively small amounts of complement proteins and immunoglobulins. The paucity of the latter two prevents effective opsonization of bacteria, an essential prerequisite for bacterial phagocytosis by neutrophils. Phagocytosis of bacteria is further impaired by the fluid nature of CSF, which is less conducive to phagocytosis than a solid tissue substrate. A critical event in the pathogenesis of bacterial meningitis is the inflammatory reaction induced by the invading bacteria. Many of the neurologic manifestations and complications of bacterial meningitis result from the immune response to the invading pathogen rather than from direct bacteria induced tissue injury. As a result, neurologic injury can progress even after the CSF has been sterilized by antibiotic therapy.

The lysis of bacteria with the subsequent release of cell-wall components into the subarachnoid space is the initial step in the induction of the inflammatory response and the formation of a purulent exudate in the subarachnoid space.

Bacterial cell-wall components, such as the lipopolysaccharide (LPS) molecules of Gram negative bacteria and teichoic acid and peptidoglycans of S. pneumoniae, induce meningeal inflammation by stimulating the production of inflammatory cytokines and chemokines by microglia, astrocytes, monocytes, microvascular endothelial cells, and CSF leukocytes.

In experimental models of meningitis, cytokines including tumor necrosis factor (TNF) and interleukin 1 (IL-1) are present in CSF within 1–2 h of intracisternal inoculation of LPS. This cytokine response is quickly followed by an increase in CSF protein concentration and leukocytosis. Chemokines (cytokines that induce chemotactic migration in leukocytes) and a variety of other proinflammatory cytokines are also produced and secreted by leukocytes and tissue cells that are stimulated by IL-1 and TNF. In addition, bacteremia and the inflammatory cytokines induce the production of excitatory amino acids, reactive oxygen and nitrogen species (free oxygen radicals, nitric oxide, and peroxynitrite), and other mediators that can induce death of brain cells.

DIAGNOSIS

Examination of CSF still offers the best chance of observing, isolating, and

identifying the causative organism in bacterial meningitis (Kaplan et al. 1986b; British Society for the Study of Infection Research Committee 1995). Lumbar puncture is particularly valuable if a dose of a parenteral antibiotic has not been given either before or on hospital admission.

The clearance of organisms in CSF after antibiotic use was estimated as 2 hrs for Meningococci and 4 hrs for Pneumococci (Kanegaye et al. 2001). Even if bacteria cannot be recovered on culture, microscopy is likely to confirm the diagnosis of bacterial meningitis and may indicate the likely causative organism. Lumbar puncture should never be used as a reason to defer commencing antibiotic treatment and other resuscitation measures. The ‘door-to-needle’ time for patients hospitalized for suspected bacterial meningitis should be less than 1 h.

DIRECT MICROSCOPY

Direct microscopy of uncentrifuged or centrifuged CSF may reveal the presence of bacteria or fungi and can provide immediate confirmation of the diagnosis. Staining with acridine orange is more sensitive than the Gram stain (Kleinman et al. 1984). The organisms seen on microscopy in antibiotic-treated patients may fail to grow on culture, but the morphology and Gram reaction of the organism, the age of the patient, and the clinical features often permit an educated guess at the identity of the causative organism.

BACTERIAL ANTIGEN DETECTION TEST

Tests for bacterial antigen in CSF can provide a quick diagnosis, but they are less sensitive than the Gram stain and do not often alter clinical management (Maxson et al. 1994). Many tests are based on the agglutination of antibody-coated latex particles and work quite well for Pneumococci, Meningococci of serogroups and for Group B Streptococci. They are less successful for the detection of serogroup B Meningococci.

The Limulus lysate test is a sensitive test for endotoxin from Gram negative organisms but has not found wide acceptance till date. Rapid identification of the offending organism by Gram's stain characteristics or bacterial antigen detection testing (BADT) allows chemoprophylaxis of contacts whenever indicated

CSF CULTURE

CSF should be inoculated on to good-quality culture media, always including at least Columbia blood agar and a heated blood agar. Plates should be incubated in five percent CO_2 for a minimum of 48 h. If a ruptured cerebral abscess is suspected, or if meningitis has followed neurosurgery or a history of previous meningeal trauma, a second blood agar plate should be incubated anaerobically for 5–7 days. If delay in processing of specimens was anticipated, CSF can be inoculated in Trans isolate medium. T-I medium is a biphasic medium that is useful for the primary culture of etiological agents of bacterial meningitis

from CSF samples. It was used as a growth medium as well as a holding and transport medium.

The possibility of parameningeal infection especially subdural empyema and brain abscess must be considered and pursued actively, if CSF shows an inflammatory response, but no bacteria is seen or cultured or detected by PCR tests and the patient's condition is not improving. CT scans may sometimes fail to detect intracranial collections of pus. Subdural empyema (or brain abscess) should be suspected if 'meningitis' is diagnosed in a child or young adult in whom there is a recent history of sinusitis or middle ear infection. The importance of not missing these conditions lies in the need for urgent neurosurgical and ear, nose, and throat (ENT) assessment as part of the management protocol. Most cases of subdural empyema are initially misdiagnosed as bacterial meningitis.

BLOOD CULTURE

For the diagnosis of bacterial meningitis, blood should be collected when a spinal tap is contraindicated or cannot be performed for technical reasons. Blood should be cultured in Trypticase soy broth (TSB) or Brain Heart Infusion with a growth supplement and chemical inhibitors such as 0.025% Sodium Polyanethol Sulfonate(SPS).

POLYMERASE CHAIN REACTION

PCR tests for the detection of meningococcal and pneumococcal DNA in CSF are now used routinely in the UK. Meningococcal PCR is specific and sensitive for the diagnosis of meningococcal meningitis (Borrow et al. 1997)

and provides serogroup information in the majority of cases. Amplification of sections of 16S ribosomal RNA, common to most species of pathogenic bacteria, may also prove to be of value (Greisen et al. 1994).

TREATMENT

Bacterial meningitis is a medical emergency. The goal is to begin antibiotic therapy within 60 min of a patient's arrival in the emergency room. Empirical antimicrobial therapy is initiated in patients with suspected bacterial meningitis before the results of CSF Gram's stain and culture are known. S. pneumoniae and N. meningitidis are the most common etiologic organisms of community-acquired bacterial meningitis. Due to the emergence of penicillin and cephalosporin resistant S. pneumoniae, empirical therapy of community acquired suspected bacterial meningitis in children and adults should include a combination of dexamethasone, a third-generation cephalosporin (e.g., ceftriaxone or cefotaxime) and Vancomycin plus Acyclovir, as HSV encephalitis is the leading disease in the differential diagnosis. Ceftriaxone or cefotaxime provide good coverage for susceptible S. pneumoniae, Group B Streptococci, and H. influenzae and adequate coverage for N. meningitidis. Cefepime is a broad spectrum fourth generation cephalosporin with in vitro activity similar to that of cefotaxime or ceftriaxone against S. pneumoniae and N. meningitidis.

Ampicillin should be added to the empirical regimen for coverage of L. monocytogenes in individuals <3 months of age, those >55 or those with suspected

impaired cell-mediated immunity because of chronic illness, organ transplantation, pregnancy, malignancy or immunosuppressive therapy. In hospital acquired meningitis, and particularly meningitis following neurosurgical procedures, Staphylococci and Gram negative organisms including P. aeruginosa are the most common etiologic organisms. In these patients, empirical therapy should include a combination of Vancomycin, Ceftazidime, Cefepime or Meropenem. Ceftazidime, Cefepime or meropenem should be substituted for Ceftriaxone or Cefotaxime in neurosurgical patients and in neutropenic patients, as ceftriaxone and cefotaxime do not provide adequate activity against CNS infection with P. aeruginosa. Meropenem is a carbapenem antibiotic that is highly active in vitro against L. monocytogenes, has been demonstrated to be effective in cases of meningitis caused by P. aeruginosa, and shows good activity against penicillin-resistant Pneumococci.

PREVENTION

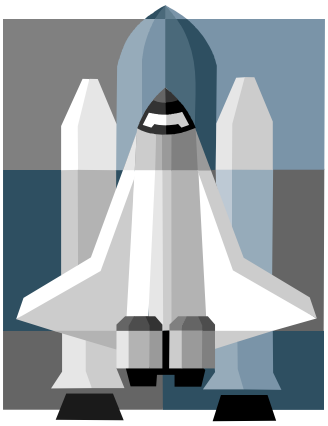
The risk of secondary cases of Meningococcal disease among close contacts (i.e. household members, day-care centre contacts, or anyone directly exposed to the patient's oral secretions) is high. Antimicrobial chemoprophylaxis with a short course of oral rifampin or a single oral dose of ciprofloxacin, or a single injection of ceftriaxone is effective in eradicating nasopharyngeal carriage of N. meningitidis. Although very effective in preventing secondary cases, antimicrobial chemoprophylaxis is not an effective intervention for altering the course of an outbreak. In epidemics, mass chemoprophylaxis is not recommended.

Vaccines have an important role in the control and prevention of bacterial meningitis. Vaccines against N. meningitidis, H. influenzae, and S. pneumoniae are currently available, but the protection afforded by each vaccine is specific to each bacterium and restricted to some of the serogroups or serotypes of each bacterium.

In industrialized countries, routine use of polysaccharide-protein Hib conjugate vaccines for immunization of infants has almost eliminated Hib meningitis and other forms of severe Hib disease. Pneumococcal polysaccharide vaccines have been used to prevent disease in the elderly and in persons with chronic illnesses that may impair their natural immunity to pneumococcal disease. Meningococcal polysaccharide vaccines are generally used in response to epidemics and for the prevention of disease in travellers although other uses are currently under investigation.

In addition to the existing armamentarium of vaccines, new generation vaccines against Meningococcal and Pneumococcal disease are under development and evaluation. These vaccines may provide a high degree of protection and broad coverage in all age groups. Until these vaccines become widely available, the current vaccines should be used appropriately and efficiently. Use of any of these vaccines will require laboratory identification of the agents causing disease in addition to epidemiological information about the age and risk groups that are most affected.

Though various Indian studies on epidemiology and comparison of various diagnostic techniques for acute bacterial meningitis are available, no such study has been carried out in Madurai. Since Government Rajaji Hospital, Madurai (GRH) is the largest tertiary care Hospital attached to Madurai Medical College catering the needs of lakhs of people from southern districts of Tamilnadu, the present study was carried out among patients admitted at Medicine & Paediatric wards at GRH and the data were analysed with reference to objectives. Efforts have been made to diagnose acute bacterial meningitis by collecting the CSF from patients satisfying the inclusion criteria and processing them by various microbiological techniques like Gram stain, culture (Direct and after Trans Isolate medium inoculation) and Latex agglutination test.



AIM & OBJECTIVES

AIM AND OBJECTIVES

The study on Prevalence and Diagnosis of Acute Bacterial Meningitis in a tertiary care centre was conducted at GRH, Madurai for a period of one year. The study was aimed

1. To understand the prevalence of Acute Bacterial Meningitis in both adult and paediatric cases admitted at GRH, Madurai
2. To identify the commonest manifestations in Acute Bacterial Meningitis.
3. To evaluate the four different techniques like Gram staining, Direct culture, TI culture, and Latex agglutination test used in the diagnosis of Acute Bacterial Meningitis.
4. To recommend the suitable methodology for the early detection of these cases to the clinicians for initiation of timely management.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Vieusseux (1805**)** described an outbreak of meningococcal disease with 33 deaths in the small community of Eaux Vives, near Lake Geneva in Switzerland, in the spring of 1805.[63] Before this, meningococcal disease may have been confused with other spotted fevers, including typhus, which occurred in clusters and outbreaks, especially in the military, and was often characterized by a hemorrhagic skin rash.

August Hirsch (1886) documented many outbreaks of infectious diseases including cerebrospinal fever in Europe and the New World up to1882. The Italian pathologists **Marchiafava and Celli** (1884) are credited with the first description of intracellular oval micrococci in a sample of CSF.[38] Three years later **Anton Weichselbaum,** in Vienna, reported the isolation of an organism, which he described as Diplococcus intracellularis meningitidis from six of eight cases of primary sporadic community acquired meningitis (Weichselbaum 1887).[65]

Eight years later came the first account of lumbar puncture in a living patient (Quincke 1893) and in 1896 meningococci were isolated for the first time from the CSF of patients with meningitis (Heubner 1896). In the same year, Meningococci were also isolated from human throat cultures (Kiefer 1896), offering an explanation for the spread of the bacteria in human populations.

Meningococci revealed more diversity than most other pathogenic human bacteria. **Frasch et al** in 1985 proposed a scheme for designation of

serotypes by which N.meningitidis is divided into 13 serogroups. Most infections are caused by A, B, C, Y and W-135. [23] **Caugant DA et al** in 1998 explained partly while analyzing the molecular epidemiology of Meningoccci by horizontal intra species recombination and incorporation from closely related Neisseria species.[11]

EPIDEMIOLOGY

Meningococcal infections occur worldwide as endemic disease[1,50].Of the five common serogroups responsible for about 90% of infections caused by Meningococci, serogroups A,B, and C account for most cases throughout the world, with serogroup A and C predominating throughout Asia and Africa and serogroups B and C responsible for the majority of cases in Europe and Americas.[1,48,50,66]

Epidemic rates varies from 1-3/100000 in many developed countries to 10-25/1,00,000 in some developing nations[7,16,44,47,57].This difference is due to difference in pathogenic properties of meningococcal strains prevalent and differences in socio economic and environmental conditions. More than half of cases among infants are caused by serogroup B for which no vaccine is available[22].

INDIAN SCENARIO

Meningococcal disease is endemic in Delhi and sporadic cases of meningitis have been occurring in Delhi in the past.[12]Isolated cases of meningitis

during 1985 were also reported from several states of India.[8]Serogroup A has been associated with all the repeated outbreaks of meningitis although serogroup B and C have been detected in a few sporadic cases.[6,28,42,54]

During 1966, 616 cases of meningitis were reported in Delhi with case fatality rate of 20.9%.The highest proportion of cases and deaths occurred in age group less than one year followed by that in 1-4 years. An outbreak of 197 cases of pyogenic meningitis with 34 deaths was reported during 1985-87[9]. N. meningitidis was the predominant pathogen isolated from 66 out of 138 CSF samples.[8,12]

A large outbreak occurred in 1985 with greater number of cases than previous years. [4] In early 2005,429 of cases of meningitis serogroup A have been reported from Delhi and adjoining areas and 128 cases have revealed microbiological evidence.[67]

In a study evaluating the epidemiological characteristics of acute bacterial meningitis in children in Egypt by **Farag HFM et al** in 2005 showed that H.influenzae(21%) was the predominant causative organism followed by S.pneumoniae(13.9%) and N.meningitidis(14-2%)[19]

Reba Kanungo et al in 2004 in a study evaluating a simple and rapid method to detect Pneumolysin in CSF for pneumococcal meningitis analyzed 75 CSF samples with presumptive diagnosis of acute pyogenic meningitis. She concluded that detection of Pneumolysin was found to be a simple low cost antigen detection assay for rapid diagnosis of pneumococcal meningitis.[46]

She did Gram stain, Culture and Pneumolysin detection by Cowan 1 staphylococcal protein A Co-agglutination technique. Pneumolysin was detected in 26(78.8%) of 33 Culture proven CSF samples and 4(9.5%) of Culture negative samples. Antigen detection by Co-A had a specificity of 90% and a sensitivity of 79% when compared with culture. Compared to Gram stain, Pneumolysin Co-A had a specificity and sensitivity of 91% and 92% respectively.

Hassib Narchi et al in 1997 in a study assessed the clinical usefulness of CSF BADT (Bacterial antigen detection test) and Gram stain in children diagnosed to have meningitis. He showed Gram stain of CSF was more sensitive than BADT (81.5% versus 57.1%) in diagnosing meningitis where the CSF culture did not show any organism (83.3% versus 50%) and also in patients who received antibiotic therapy prior to diagnosis (90% versus 50%).[26] BADT is positive only in the presence of specific polysaccharide surface antigens for Haemophilus influenzae type b, Streptococcus pneumoniae, Escherichia coli K1, group B Streptococcus and Neisseria meningitidis groups A, B, C, Y and W135 antigens, while any other bacteria remain undetected. Gram's stain does not suffer from this limitation.

In the studies done by **Feuerborn SA, Finlay FO and Maxson S,** they did not recommend routine BADT in all CSF studies, but only in cases where Gram's stain did not identify any organism or if it suggested a Meningococcal infection.[20,21,40]

Susan K. Smith et al in 1984 conducted a study to evaluate the rapid Latex slide agglutination test for identification of Pneumococci. He concluded that Latex test proved to be a rapid and accurate technique for identifying S.pneumoniae, provided care was taken to distinguish between agglutination and clumping of latex particles. [56]

Sherry A. Dunbar et al (1998) in a study evaluated microscopic examination and broth culture of CSF in the diagnosis of meningitis. He showed that microscopic examination of Gram stained CSF detected the correct etiologic agent in 88% of bacterial meningitis samples.[53] And if the samples collected from the pretreated cases were excluded, the sensitivity increased to 92%. Microscopic examination incorrectly suggested the presence of organisms in only 3 of 2,635 (0.1%) CSF examinations. Thus, microscopic examination of Gram stained, concentrated CSF is highly sensitive and specific in early diagnosis of bacterial meningitis.

Chapin-Robertson et al and **Shanholtzer et al** had showed that sensitivity of Gram stain examination was increased by concentrated smears prepared with a cytospin centrifuge.[14,52]

Acute bacterial meningitis remains a major cause of mortality and long term neurological sequelae worldwide. Despite the availability of potent newer antibiotics, the mortality rate remains significantly high in India and other developing countries, ranging from 16-32%[5,15,31,58]. There is a need for a periodic

review of bacterial meningitis worldwide, since the pathogens responsible for the infection vary with time, geography and patient age[58].

A study by **Vande Beek, de GANS et al** in 2006, showed that any delay in the diagnosis and initiation of antimicrobial therapy can result in poor outcome of the disease [62]. Since clinical signs of meningitis cannot always be relied upon, laboratory support is imperative to achieve an early diagnosis[15].

In a retrospective study done by **Mani R et al** in 2007 at NIMHANS Bangalore, Bacteriological spectrum of Community Acquired Acute Bacterial Meningitis of 385 patients were analysed.[36] The bacterial pathogens could be demonstrated by cytospin Gram stain in CSF samples of 253(65.7%) patients, while 157(40.8%) samples yielded growth on Culture. An additional 122 cases of Culture negative cases could be identified by LAT (62 cases) and typical morphology on Gram stain (60 cases). Of the 62 samples positive by LAT, 53 samples were also positive by Gram stain, while nine samples were smear negative. In the 60 samples where bacteria (pneumococci) could be identified only by typical Gram stain morphology, LAT was negative in 23 cases, while it could not be done in remaining. The high yield of pathogens on Gram stain can be attributed to routine use of cytospin to concentrate the smear.

A cytospin also provides several diagnostic benefits like good preservation of morphology of cells and bacteria and an increased rate of detection of bacterial pathogens especially in partially treated pyogenic meningitis, which can mimic Tuberculous Meningitis posing a diagnostic dilemma for clinicians.[36]

This study showed Streptococcus pneumoniae in 238 cases (61.8%) and it was the major etiological agent of Acute Bacterial Meningitis both in adults and children and it reflected a similar trend reported in an earlier study from the same institute (1978-1988).[13] **Chinchankar N et al** and **Kabra SK et al**, in their studies reported a high incidence of Pneumococcal meningitis.[15,31]

In recent years, Group B Streptococcal meningitis known to cause meningitis in neonates, is being increasingly recognized as a cause of meningitis in adults in Southeast Asia and is associated with a high case fatality.[68]

Studies done by **Chinchankar N et al** and **Sherry A Dunbar et al**, reported that the sensitivity and specificity of CSF Gram stain was 60-90% and >97% respectively, stressing its importance in the rapid and accurate diagnosis of the causative bacteria.[15,53]

In a study by **Van de Beek et al** in 2004, patients with Pneumococcal meningitis were found to be at risk for an unfavorable outcome, even after correction for other clinical parameters . He stressed the importance of need for clinicians to know the causative organism in predicting the outcome by Gram stain as a routine procedure for prompt identification of the pathogen.[61]

In their studies, **Chinchankar N et al**, **Das BK et al** and **Kabra SK et al** reported a low CSF culture positivity, ranging from 6-50%.[15,18,31] Various reasons cited in the literature for the low yield of bacteria on culture were prior antibiotic therapy, delay in transport of specimens to the laboratory, non availability of special media for specific pathogens, presence of autolytic enzymes

in CSF samples. The need for development of Neuromicrobiology especially in neurological/neurosurgical[13] centres, encouraging a careful Gram staining and prompt bed side inoculation of CSF on culture media by resident doctors had been stressed.[31]

Relating to Latex agglutination test (LAT), **Perkins et al** in 1995 in a study to determine the efficacy of antigen detection tests did not identify any false negative LAT suggesting a high sensitivity of these tests.[43] However a similar Indian study had reported a sensitivity of 83% .[18]

LAT kits does not detect all the capsular serotypes prevalent in our geographical area or yet unrecognized causative agents. Some workers have questioned the clinical usefulness of antigen detection tests.[33] explaining that a negative test does not rule out infection and false positive results may lead to unnecessary prolonged course of antibiotics , lengthened hospital stay and in some cases important clinical implications.

Recent immunization with Hib conjugate vaccine and infection with cross reacting organisms were quoted as common reasons for false positive LAT. However, several studies[15,18] advocate the usefulness of LAT, especially in pretreated cases and to differentiate partially treated pyogenic meningitis from Tuberculous meningitis which is rampant in India. Despite its drawbacks, LAT is a simple and rapid procedure suitable to be an adjunct laboratory test, but needs to be interpreted cautiously taking in to account the patient's clinical condition.

In a study, **Surinder K et al** in 2007 reviewed the results of microscopic examination, routine culture and antigen detection by latex particle agglutination test of 65 patients in order to establish the diagnostic value of LAT for the aetiological diagnosis of bacterial meningitis. CSF culture was positive in only 15(23.1%) cases. Of these 15 samples, the Gram stain showed positive results in 11(73.3%) and LAT detected bacterial antigens in 10(15.4%) samples. The results demonstrated that LAT positivity correlated well with culture results and they concluded that it can be included as an adjunct laboratory test.[55]

In a study, **John T Kanegaye et al** in 2001 reviewed 128children with bacterial meningitis to describe the rate at which parenteral antibiotic pretreatment sterilizes CSF cultures. The decay in yield of CSF cultures over time was evaluated in patients with lumbar punctures (LP) delayed until after initiation of parenteral antibiotics and in patients with serial LPs before and after initiation of parenteral antibiotics. Thirtynine patients (30%) had first LPs after initiation of parenteral antibiotics, and 55 (43%) had serial LPs before and after initiation of parenteral antibiotics.[29]

After >50 mg/kg of a third-generation cephalosporin,3 of 9 LPs in meningococcal meningitis were sterile within 1 hour, occurring as early as 15 minutes, and all were sterile by 2 hours. With pneumococcal disease, the first negative CSF culture occurred at 4.3 hours, with 5 of7 cultures negative from 4 to 10 hours after initiation of parenteral antibiotics. Group B streptococcal cultures were positive through the first 8 hours after parenteral antibiotics. This study

demonstrated that CSF sterilization may occur more rapidly after initiation of parenteral antibiotics than previously suggested, with complete sterilization of meningococcus within 2 hours and the beginning of sterilization of pneumococcus by 4 hours into therapy.

Viswanath G et al in 2007 carried out a study to determine the prevalence of pyogenic meningitis in children and to find out the sensitivity of Gram stain, CRP and Latex agglutination tests for the diagnosis of pyogenic meningitis from CSF samples.[64] Out of 150 CSF samples studied, 40 were diagnosed as lab proven pyogenic meningitis samples and among them H. influenzae was the commonest organism (22.5%) isolated. The sensitivity of Gram stain was 89% and was more than that of **Gaitonde et al**[24] 68%, **Lidia et al**[35] 81% and **Rao B N et al** [45]86%. The sensitivity of Latex agglutination test was 89% and it was less than that of **William et al** who have reported 100% sensitivity.[69] As most of the cases included in the study were treated earlier, the culture positivity was only 62.5%. On conclusion, Gram stain and/or Latex agglutination tests, if done properly are the most rapid and reliable tests for the diagnosis of pyogenic meningitis.

Sameer Marji et al in 2007 in a study reviewed the bacteriological profile of bacterial meningitis in children .The medical records of 50 children with the diagnosis of bacterial meningitis during 4 years period, were reviewed. The main cause of infection was Streptococcus pneumoniae followed by Haemophilus influenzae and Neisseria meningitidis.[49] Mortality was higher in infants and in

Meningococcal infection, while complications were more encountered in cases of Streptococcus pneumoniae. Cerebrospinal fluid culture was positive in 11 samples and Latex agglutination test was positive in 39 samples.

Komolpis P et al in 1995 did a comparative study using the Latex particle agglutination test (LPAT) in cerebrospinal fluid for the diagnosis of bacterial meningitis with CSF culture as gold standard. 299 children, ranging from 3 months to 14 years of age were included in this investigation. One hundred and forty-four presented a positive CSF Culture. The sensitivity and the specificity of LPAT was 95.7and 100.0% for N. meningitidis, 95.2 and 100.0% for H. influenzae type b and 86.5 and 100% for S.pneumoniae respectively. When all three organisms were considered simultaneously, the sensitivity and the specificity was 93.0 and 100.0%, respectively. LPAT was thus a useful diagnostic test for bacterial meningitis, especially in developing countries where laboratory facilities are limited.[33]

Kaiser Tarafdar et al in 2001[32] evaluated the sensitivity of the Latex agglutination test as one of the available methods of CSF bacterial antigen detection in the samples which were negative by both Gram stain and Culture. The study showed that the sensitivity of the latex agglutination test for detection of bacterial antigen in the CSF among the culture negative samples was only 7%.

Alka E. Sonavane et al in a study done at Mumbai in 2008 showed that Streptococcus pneumoniae was the commonest isolate (12cases).[3]

Hoban DJ et al in 1985[27] in a study compared the sensitivity and specificity of four test systems in detecting Haemophilus influenzae type b, Neisseria meningitidis, Streptococcus pneumoniae, and Gram negative organisms versus Culture in CSF samples. The tests used on CSF from 155 patients with meningitis were the Phadebact Coagglutination (CoA) test, the Directigen Latex agglutination (LA) test, Counterimmunoelectrophoresis (CIE), and the Limulus amebocyte lysate (LAL) test. The sensitivity of LAT was 78% for detection of H.influenzae, 100% for S.pneumoniae and 33% for detection of N.meningitidis. The detection of bacterial antigen from CSF in patients with meningitis by agglutination tests is more sensitive and highly specific.

MATERIALS & METHODS

MATERIALS AND METHODS

This study was conducted at Government Rajaji Hospital, attached to Madurai Medical College, Madurai and the CSF samples were obtained from patients admitted in the Department of Medicine and the Department of Paediatrics. The study period was for one year from Feb 2008 to Jan 2009 and the study population included 200 suspected cases of acute bacterial meningitis with fever, vomiting and headache. Ethical committee clearance was obtained prior to the onset of the study and informed consent was obtained from each participant.

The inclusion criteria for the study population were

Acute febrile illness with the following symptoms.

a. Head ache
b. Vomiting
c. Altered sensorium
d. Neck rigidity
e. Rash
f. Hemorrhagic manifestations
g. Refusal of feeds
h. Seizures

The exclusion criteria were

a. Febrile cases without the above symptoms
b. Patients with recurrent meningitis due to structural defects in CNS
c. Tuberculous meningitis.

COLLECTION OF CEREBROSPINAL FLUID (CSF)

CSF samples were collected by lumbar puncture.

Lumbar Puncture

The kit for collection of CSF contains:

1. Skin disinfectant 2. Sterile gauze and Band-Aid 3. Lumbar puncture needles: 22 gauge/3.5"for adults; 23 gauge/2.5" for children, 4. Sterile screw-cap tubes, 5. Syringes and needles, 6. Transport container, 7. Trans-Isolate (T-I) medium (as CSF cannot be analyzed in the microbiological laboratory immediately).

The patient was kept in either sitting up or lying on the side, with his or her back arched forward so that the head almost touches the knees during the procedure. The skin was disinfected along a line drawn between the crests of the two ilia with 70 % alcohol to clean the surface and remove debris and oils. Then tincture of iodine or povidone-iodine was applied. After drying, the needle was introduced, and the drops of fluid (1 ml minimum, 3-4 ml if possible) were collected into sterile, screw-cap tubes and also inoculated in Trans isolate medium.

After proper labeling, it was carried to the laboratory as soon as possible avoiding exposure to excessive heat or sunlight.

TRANSPORT OF CSF SPECIMENS

S. pneumoniae, H. influenzae and N. meningitidis are fastidious and fragile bacteria. As soon as the CSF had been collected, it was transported to the microbiology laboratory, where it was examined as soon as possible (within one hour from the time of collection). It was not exposed to sunlight or extreme heat or cold. If delay in processing of specimens was anticipated, CSF was inoculated in Trans isolate medium.

T-I medium is a biphasic medium that is useful for the primary culture of etiological agents of bacterial meningitis from CSF samples. It was used as a growth medium as well as a holding and transport medium.

T-I MEDIUM CONSISTS OF

1. Solid phase: Activated charcoal, Soluble Starch, Agar.

2. Liquid phase: Tryptic soy broth, Gelatin, MOPS (N-morpholino propanesulfonic acid) buffer.

T-I MEDIUM INOCULATION

(a) The T-I bottle septum was disinfected with alcohol and iodine and allowed to dry before inoculation. 1 ml of CSF was inoculated into the T-I medium, which was either pre-warmed in the incubator (35 ° C-37 ° C) or kept at room temperature 25 ° C .

The remaining CSF was kept in the container in which it was collected. It was not refrigerated, but held at room temperature before Gram staining.

(b) After inoculation, the T-I bottle was labeled appropriately with the patient's identification, date and time of CSF inoculation and transported to the lab. In the laboratory, the media was vented with a sterile cotton-plugged hypodermic needle. Then the T-I medium was incubated at 35 ° C for up to 7 days.

PRIMARY CULTURE AND PRESUMPTIVE IDENTIFICATION

A. Inoculation of Primary Culture Media

Once the CSF has arrived at the microbiology laboratory, it was centrifuged for 20 minutes at 2000 rpm. Supernatant was drawn off with a Pasteur pipette and used for antigen detection by latex agglutination. Sediment was either vigorously vortexed or well mixed. One or two drops of the sediment were used to prepare the Gram stain and one drop to streak each of the primary culture media (Blood Agar, Chocolate Agar, Chocolate Agar with IsoVitaleX, MacConkey Agar and Nutrient Agar plates). If less than 1 ml of CSF was available, it was not centrifuged but CSF itself was used for Gram staining and plating.

BAP, which is a trypticase soy agar (TSA) plate containing sheep blood (5%), was used for the isolation of S.pneumoniae. For H. influenzae, a Chocolate Agar plate supplemented with a growth supplement IsoVitaleX was used. BAP and CAP were used for N. meningitidis. The agar plates were incubated in a 5% CO_2 incubator.

100 microlitre of the liquid portion of the culture material which was already inoculated in TI medium and incubated for 24 hours were inoculated on the NAP ,BAP, CAP, Macconkey plates as above and the plates were incubated overnight.

A presumptive diagnosis of bacterial meningitis caused by H. influenzae, S. pneumoniae, and N. meningitidis, Group B Streptococci, E.coli were made by Gram stain of the CSF sediment or by detection of specific antigens in the CSF by the Latex agglutination test. The positive results of either or both tests provided evidence of infection, even if cultures failed to grow.

GRAM STAINING

(a) CSF was centrifuged for 20 minutes at 2000 rpm.

(b) The smear was prepared by placing 1 or 2 drops of sediment on an alcohol-rinsed and dried slide, allowing drop(s) to form one large drop and the smear was not spreaded out.

(c) The slide was air dried in the biosafety cabinet

(d) Then the slide was fixed with methanol (95%).

(e) The smear was flooded with methyl violet and allowed to stand for 1 minute.

(f) It was rinsed gently with tap water and the excess water was drained.

(g) The smear was then flooded with Gram's iodine solution and was allowed to stand for 1 minute.

(h) It was rinsed gently with tap water and drained.

(i) Decolorization was done with acetone.

(j) Counter staining with dilute carbol-fuchsin for 30 seconds was done.

(k) The slide was rinsed with tap water and air dried.

(l) The stained smear was examined microscopically, using a bright-field condenser and an oil-immersion lens.

APPEARANCE IN GRAM STAIN	ORGANISM INFERRED
Gram positive lanceolate shaped diplococci	**Streptococcus pneumoniae**
Gram positive cocci in chains	**Group B Streptococcus**
Gram negative intra cellular diplococci	**Neisseria meningitidis**
Gram negative bacilli	**E.coli**
Gram negative long filamentous bacilli	**H.influenzae**

General Method for Performing Latex Agglutination Tests

The manufacturer's instructions were precisely followed when using these tests. General recommendations and instructions typical for the detection of soluble bacterial antigens were followed. The supernatant of the centrifuged CSF sample was tested as soon as possible. Reagents were kept refrigerated between 2 ° C and 8 ° C when not in use.

Performance of the Test

(a) The supernatant of the CSF was heated in a boiling water bath for 5 minutes.

(b)After shaking the latex suspension , one drop of each latex suspension was placed on the disposable card.

(c) Then 30-50 µl of the CSF was added to each suspension.

(d) The card was rotated by hand for 2-10 minutes.

The test results were read under a bright light without magnification.

Positive reaction: Agglutination (or visible clumping) of the latex particles occurring within 2 minutes.

Negative reaction- Homogenous and slightly milky suspension.

CULTURE IDENTIFICATION

The morphology of colonies was observed on the plates. Based on the colonial morphology and other identification tests(Oxidase ,Bile solubility, Optochin sensitivity, CHO Utilization test, Factor X and V Requirement, CAMP test) isolates were identified and confirmed.

COLONIAL MORPHOLOGICAL IDENTIFICATION

Large, flat, colorless to grey opaque colonies with no haemolysis and discoloration of the medium were identified as H.influenzae. H. influenzae was further confirmed by Oxidase test and Factor X and V requirements.

Round, smooth, moist, glistening and convex,with an entire edge, greyish and unpigmented were identified as N.meningitidis. The growth was confirmed with Oxidase and Carbohydrate Utilization tests.

Small, greyish, moist colonies with a greenish zone of alpha-haemolysis on BAP were identified as S. pneumoniae. Young pneumococcal colonies appeared raised which became flattened after 24 hours to 48 hours with the central portion being depressed. Further identification was done with Bile solubility, Optochin

susceptibility and Inulin fermentation tests and Intra peritoneal inoculation of mice.

Colonies that were grey, mucoid and large (about 2 mm) with Beta hemolysis on blood agar plate were identified as Group B Streptococci. The growth was further identified by CAMP reaction and Hippurate hydrolysis test.

Large, smooth, moist,opaque to partially translucent,hemolytic and lactose fermenting colonies were identified as Escherichia coli.

GROWTH ON		**Gram stain**	**Identification**
CAP	**BAP**		
+	+	**Gram negative diplococci**	**N.meningitidis**
+	+	**Gram positive diplococci**	**S.pneumoniae**
+	-	**Gram negative pleomorphic coccobacilli**	**H.influenzae**
+	+	**Gram positive cocci in chains**	**Gr B Streptococci**

IDENTIFICATION TESTS:

A. Kovac's Oxidase Test

The oxidase test determined the presence of cytochrome oxidase. The reagent tetramethyl-p-phenylenediamine hydrochloride was turned into a purple compound by organisms containing cytochrome c as part of their respiratory chain.

Performance of the Test

Using a disposable plastic loop, a portion of the colony was picked and rubbed onto a Oxidase disc.

Reading the Test Results

The appearance of purple colour within 10 seconds was taken as positive.

B. **Carbohydrate Utilization by N. meningitidis - Cystine Trypticase Agar Method:**

To confirm a culture as N. meningitidis, a set of four tubes, each containing a sugar (glucose, maltose, lactose, and sucrose to a final concentration of 1%) were used. N. meningitidis oxidized glucose and maltose, but not lactose and sucrose.

A phenol red indicator included in the medium developed yellow colour in the presence of acid,at a pH of 6.8 or less.

Performance of the Test

(a) A small amount of growth from an overnight culture on BAP or CAP was taken with a sterile inoculating needle.

(b) The inoculum was stabbed several times into the upper 10 mm of medium.

(c) Caps of tubes were tightly closed and placed in a 35°C incubator (without CO2) and incubated for at least 72 hours before discarding as negative.

Reading the Test Results

Development of visible turbidity and a yellow colour in the upper portion of the medium indicates growth and the production of acid and was interpreted as a positive test.

C. Bile Solubility Test

Performance of the Tests

(a) A loop of the strain was taken from the growth on a BAP and a suspension was made in 0.5 ml of sterile saline. The suspension was compared with 0.5 McFarland density standard.

(b) The suspension was divided into two equal amounts (0.25 ml per tube), and 0.25 ml of saline was added to one tube and 0.25 ml of 2 % deoxycholate (bile salts) to the other. The tubes were gently shaked and incubated at 35 ° C-37 ° C for 2 hours.

(c) The tubes were inspected periodically for lysis of cells in the tube containing the bile salts. A clearing or a loss of turbidity of the broth, was taken as positive result.

Alternatively, for testing bile solubility by plate method, a drop of solution of 10% sodium deoxycholate was placed directly on a colony and the plate was kept at room temperature or in an aerobic incubator at 35C for approximately 15 minutes. The disappearance of the colony was taken as positive.

D.Susceptibility to Optochin

Performance of the Test

(a) The suspected alpha-haemolytic colony was touched with a sterile bacteriological loop and streaked onto a BAP.

(b) An optochin or "p" disk with a diameter of 6 mm (containing 5 µg ethylhydrocupreine) was placed aseptically on the end of the streak where the loop was first placed.

(c) Then the plates were incubated in a CO2 incubator or candle-jar at 35 ° C for 18-24 hours.

Reading the Test Results

Alpha-haemolytic strains with a zone of inhibition of growth greater than 14 mm diameter were considered as Pneumococci.

Interpreting the Test Results

The identification of Pneumococci based on the Optochin and bile solubility tests was as follows:

(a) A strain showing a zone of inhibition by Optochin of 14 mm or more was identified as Pneumococcus.

(b) A strain showing a smaller but definite zone of inhibition by Optochin and was bile soluble, was also identified as Pneumococcus.

E.IDENTIFICATION OF X AND V FACTOR REQUIREMENTS

H. influenzae is a fastidious organism requiring media containing Haemin(X factor) and Nicotinamide adenine dinucleotide (NAD,Vfactor). H.influenzae was identified on the basis of its growth requirements for X and V factors.

Performance of the Test

(a)A heavy suspension of cells was prepared (No. 1 McFarland) from a primary isolation plate in peptone water.

(b) A sterile swab of the suspension was streaked over one-half of Heart infusion plate, and disks containing X, V, and XV factors were placed on the inoculated plate after the inoculum had dried.

Reading the Test Results

Growth around the discs containing X and V factors confirmed the H.influenzae isolates.

F.CAMP (Christie, Atkins, Munch, Peterson) test

A known Beta-hemolytic Staphylococcus strain was streaked across the blood agar plate. Then the test organism was inoculated at right angles to it without touching the Staphylococcal inoculums. An Enterococcal species was inoculated as a negative control. The plate was then incubated at 37 C.

READING OF THE RESULT

An arrow head shaped enhancement in hemolysis where the Staphylococcal organism met the test organism was taken as positive.

G. INTRA-PERITONEAL INOCULATION OF MICE:

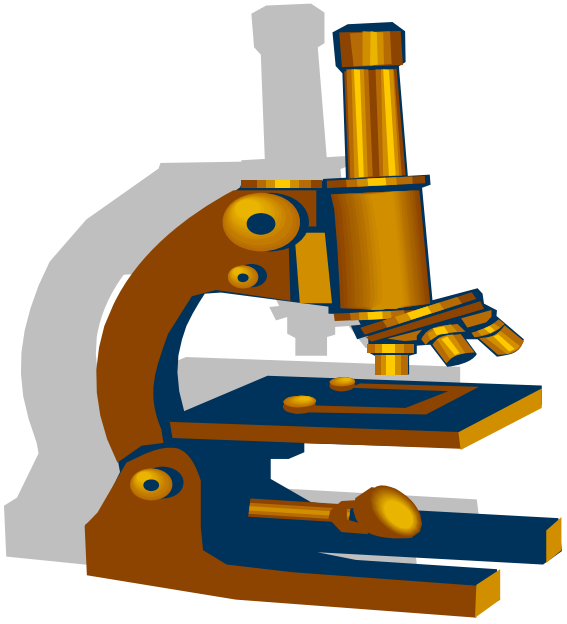
1. The mouse was anaesthetised.

2. Using a 10 x 0.5mm hypodermic needle, 1 ml of the inoculums was injected intra-peritonealy without going deep and lacerating the abd.organs and great vessels.

3. The mouse was released while the needle was withdrawn.

4. Organisms were demonstrated in the heart blood by Gram staining and Culture after 24 to 48 hours.

Gram stain	Latex agglutination	Growth on bap	cap	na	mac	Colonial morphology	Oxidase test	CHO utilization/fermentation	Factor x,vrequirement	Bile solubility test	Optochin sensitivity test	Inulin fermentation Hipp.hydrolysis	CAMP test	Animal inoculation	Organism identified
Gram pos lanceolate shaped diplococci-	POS for S.pneumonia	+	+	–	–	Alpha hemolytic in BAP	Neg	G-+ M-+ L - - S - -	Neg	Pos	Sensitive	+ (Intraperitoneal inoculation of mice)	–	+	Streptococcus .pnemonia
Gram pos cocci in chains	POS for Gp B Strep	+	+	–	–	Beta hemolysis	Neg	-	Neg	Neg	-	–	+		Group B Streptococci
Gram neg intra cellular diplococci	POS for N.meningitidis	+	+	–	–	Weak hemolysis	Pos	-	Neg	Neg	-	–	–		Neisseria meningitidis
Gram neg bacilli	POS for E.coli K1	+	+	+	+	hemolytic	Neg	G-+ L-+ S- +	Neg	Neg	-	–	–		Escherichia coli K1
Gram neg long filamentous bacilli	Pos for H.influenzae	–	+	–	–	Gamma hemolysis	Pos	-	Pos	Neg	-	–	–		Hemophilus. influenzae



RESULTS

\RESULTS

CSF Samples of 200 patients who were admitted as suspected cases of acute pyogenic meningitis in the medical and the paediatric wards at Govt. Rajaji Hospital, Madurai were analyzed in this study. The age group involved in paediatric cases were 0-12 years and adult cases were more than 12 years. All CSF samples were subjected to Gram staining, Culture (Direct culture and Trans Isolate Medium culture) and Latex agglutination test.

On analyzing the 200 cases, it was found that 113 were males (56.5%) and 87 were females (43.5%) showing high **incidence of acute pyogenic meningitis in males.** The cases were further analysed agewise and sexwise and it was found that among the 113 males, 49 cases were in the age group of 0-6 years (43%), 29 were in the age group of 7-12 years (26%),10 were in the age group of13-18 years (5%), 8 were in the age group of 19-24 years (4%), 5 were in the age group of 25-30 years (1%) and 4 were in the age group of 31-36 years (2%). Similarly among 87 females, 27 were in the age group of 0-6 (31%),18 were in the age group of 7-12(21%), 9 were in the age group of 13-18(4.5%), 9 were in the age group of 19-24(4.5%), 5 were in the age group of 25-30 (2.5%) years and 4 were in the age group of 31-36 years (2%).This is shown in Table no. 1

Table –1 AGE AND SEX DISTRIBUTION OF STUDY SUBJECTS

Sl. No.	Age Group	Male	Female	TOTAL n=200
1	**0-6**	**49**	**27**	**76(38%)**
2	7-12	29	18	47(23.5%)
3	13-18	10	9	19(9.5%)
4	19-24	8	9	17(8.5%)
5	25-30	2	5	7(3.5%)
6	31-36	4	4	8(4%)
7	37-42	3	11	14(7%)
8	43&above	8	4	12(6%)
TOTAL		113(56.5%)	87(43.5%)	200(100%)

It was found that the age group commonly involved in both sexes were between 0-6 years.

The cases were further analysed according to clinical features and it was found that all cases presented with fever (100%), 141 cases with altered sensorium (71%), 137 cases with vomiting (69%), 108 cases with headache(54%), 103 cases with neck rigitidy , 36 cases with seizure (18%) and 14 cases presented with skin rash (7%). Skin rash as a symptom was seen in patients above 12 years. This is depicted in Table no 2.

TABLE 2 - ACUTE PYOGENIC MENINGITIS Vs CLINICAL FEATURES

CLINICAL FEATURES	0– 12 years (paediatric group) n=123	> 12 years (adult group) n=77	Total n=200
FEVER	**123**	**77**	**200 (100%)**
ALTERED SENSORIUM	93	48	141(71%)
VOMITING	81	56	137 (69%)
HEADACHE	42	66	108(54%)
NECK RIGIDITY	57	46	103(52%)
REFUSAL OF FEEDS	44	0	44(22%)
SEIZURES	28	8	36(18%)
SKIN RASH	0	12	12(6%)

It was found that fever was invariably present in all cases irrespective of age and sex. Refusal of feeds was markedly noted in paediatric cases.

On testing the 200 CSF samples taken from 200 patients, thirty one samples(15.5%) were lab proven positives for acute pyogenic meningitis. The samples which showed positive by any one methodology along with clinical symptoms of meningitis were taken as lab proven positives. The positive samples were further analysed age and sex wise and it was found that among the 31 positive acute bacterial meningitis cases, nineteen cases (61%) were in the

paediatric age group (<12 years) and twelve cases (39%) were in adult group. Of the nineteen paediatric patients, twelve (63%) children were males and seven (37%) were females and among twelve adult patients, seven (58%) were males and five (42%) were females. The results are shown in TABLE 3.

Table –3 AGE AND SEX WISE DISTRIBUTION OF POSITIVES

AGE IN YEARS	MALES	FEMALES	TOTAL
0-6	**8**	**3**	**11(35.5%)**
7-12	4	4	8(26%)
13-18	1	1	2(6.5%)
19-24	3	2	5(16%)
25-30	2	2	4(13%)
31-36	1		1(3%)
37-42	-	-	
43 & above	-	-	
TOTAL	19(61%)	12(39%)	31(100))%

Thus, the analysis of samples also showed that the common age group affected in the lab proven acute bacterial meningitis in this study was 0-6 years and males were most commonly affected .

All the 200 samples were processed by Gram staining, Culture (Direct and Trans isolate medium) and Latex agglutination test. Among the 200 samples, 31 samples showed positive results.

Among the 31 lab proven positives, Gram staining showed the presence of organisms in 25 samples (81%). Of the 25 positive samples, 17 were Gram positive and 8 were Gram negative. Among the Gram positive, all 17 were cocci and among Gram negative 3 were cocci and 5 were bacilli. This is given in Table 4.

TABLE 4 GRAM REACTION OF POSITIVE ISOLATES

Gram reaction	Cocci n=20	Bacilli n=5	Total n=31
Gram positive	**17(55%)**	**-**	**17(55%)**
Gram negative	**3(10%)**	**5(16%)**	**8(26%)**
No reaction	**-**	**-**	**6(19%)**

It was noted that Gram positive cocci were more detected by Gram staining technique.

All the 200 samples were directly cultured on Nutrient agar plate , Mac Conkey agar plate, Blood agar plate, Chocolate agar plate and Chocolate agar with IsoVitaleX, and it was found that 12 samples(6%) were positive for various organisms. Similarly all the 200 samples incubated in Trans isolate medium were

also cultured on these plates and it was found that 18 (9%) were positive for various organisms

TABLE 5.REPORT OF DIRECT AND TI MEDIUM CULTURES.

No of organisms cultured	No. of isolates By Direct culture	No. Of Isolates By culture With TI Medium
200	**12(6%)**	**18(9%)**

It was obvious that the number of organisms isolated in TI medium were more when compared with direct culture.

Out of the 31 lab proven meningitis samples, Direct culture yielded growth in 12 (39%) samples and TI culture isolated organisms in 18 (58%) samples. All the 12 cultures isolated by Direct culture method were analysed for the organisms isolated and it was found that 5 were S.pneumoniae, one each was N.meningitidis and Gr B Streptococcus, two were E.coli K1and two were Klebsiella and one was S.aureus. Similarly all the 18 cultured in TI medium were analysed for organisms and it was found that 9 were S.pneumoniae, 2 each were N.meningitidis , E.coli K1 and Klebsiella, one each was H.influenzae, Gr B Streptococcus and S.aureus. This is given in the Table. 6

TABLE 6 ORGANISMWISE COMPARISON BETWEEN CULTURE METHODS

ORGANISMS DETECTED	DIRECT CULTURE Total=31	T I CULTURE Total=31
S. pneumoniae	**5**	**9**
N. meningitidis	**1**	**2**
H.influenzae	**0**	**1**
Gr B Streptococcus	1	1
Escherichia coli K 1	2	2
Others	3	3
Total	12 (39%)	18 (58%)

It was noted that more no. of S.pneumoniae, N.meningitidis, H.influenzae were isolated in TI medium whereas the other organisms were isolated equally in both media. Thus it was proved **that culture in T.I medium was a better method for isolation of fastidious pathogens causing Acute Pyogenic Meningitis.**

All the 200 samples were further processed with Latex agglutination test by Wellcogen Kit and 26 out of 200 [13 %] were positive for various organisms. Of the 31positive samples, 26 (84%) were detected by Latex agglutination test. Out of 26 positive samples, 18 (69%) were positive for S.pneumoniae, three (11%) were positive for H.influenzae and two [8%] were positive for both N.meningitidis and E.coli K 1 and one(4%) was positive for Gr. B Streptococcus .This is given in Table.7

TABLE 7 REPORT OF LATEX AGGLUTINATION TEST

Organisms isolated	No.of samples identified by LAT [n=26]	No of samples not identified by LAT[n=5]
S. pneumoniae	18(58%)	1
N. meningitidis	2(6.5%)	1
H.influenzae	3(10%)	-
Gr B Streptococcus	1(3%)	-
Escherichia coli K 1	2(6.5%)	-
Others	-	3
Total	26(84%)	5(16%)

It was noted that Latex agglutination detected only the specified pathogens and among them more number of S.pneumoniae were detected by Latex agglutination.

The samples were further analysed as per the organisms isolated. It was found that Streptococcus pneumoniae was the most common pathogen isolated from 19(61%) samples which included 13 samples from paediatric cases and 6 from adult patients. Neisseria meningitidis was isolated from 3(10%) adult samples. Hemophilus influenzae was isolated in 3(10%) paediatric samples. Escherichia coli K1 was isolated one each (3%) from adult and paediatric samples. Group B Streptococci was isolated from one (3%) paediatric sample. Other Non

fastidious bacteria, Klebsiella pneumoniae and Staphylococcus aureus were isolated from two (6.5%) and one(3%) sample respectively. This is shown in Table 8.

TABLE –8: COMPARISON OF ORGANISMS IN PAEDIATRIC AND ADULT GROUPS

SPECIES	0– 12 years (paediatric group)	> 12 years (adult group)	Total n=31
S. pneumoniae	**13**	**6**	**19(61%)**
N. meningitidis	-	3	3(10%)
H.influenzae	3	-	3(10%)
Gr B Streptococcus	1	-	1(3%)
E. coli K1	1	1	2(6.5%)
Staph.aureus	-	1	1(3%)
K. pneumoniae	1	1	2(6.5%)
Total	**19**	**12**	**31**

It was noted that S.pneumoniae was isolated in all age groups whereas N. meningitidis was isolated only in adults more than 12years and H.influenzae, Gr B Streptococci only in paediatric age group.

The four techniques (Gram staining, Direct culture, TI culture, Latex agglutination test) involved in the diagnosis of organisms causing acute pyogenic meningitis were compared and the results were analysed. It was noted that out of

200 samples processed, 25(12.5%) were positive by Gram staining, 12(6%) were positive by Direct culture, 18(9%) by TI medium and 26(13%) were positive by Latex test. This is given in Table.9.

TABLE 9 DETECTION BY VARIOUS TECHNIQUES

No. of positives	Gram staining	Direct culture	TI culture	Latex test
31	25(81%)	12(39%)	18(58%)	26(84%)

It was noted that Latex agglutination and Gram staining techniques identified the organisms more or less equally. Among the cultures, more organisms were isolated in TI media culture than direct culture.

On comparing Gram staining with culture, it was shown that 25 out of 31 positives (81%) showed the presence of organisms by Gram staining while only 18 (58%) samples yielded growth by culture. Among the 25 smear positive samples, only 15 samples showed growth in culture and 10 samples were culture negative. Of the 6 smear negative samples, 3 were culture negative and 3 samples yielded pathogens in culture. This is shown in Table 10.

TABLE 10 CORRELATION OF SMEAR AND CULTURE IN PYOGENIC MENINGITIS

	Culture +	Culture -	Total
Smear positive	15	10	25
Smear negative	3	3	6
Total	18	13	31

It may therefore imply that a simple Gram stain would afford more information on the causative pathogen in meningitis and isolation would confirm the diagnosis, although in some cases culture was negative. Use of TI medium helped to recover fastidious organisms as compared to conventional culture.

On comparing Gram staining with Latex, it was found that LAT was positive for 26 and Gram staining showed the presence of organisms in 25 samples out of the total 31 lab proven acute bacterial meningitis samples. Among the 26 LAT positives, Gram staining identified organisms in only 20 samples. And among the 5 LAT negative samples, Gram staining technique showed the presence of organisms in all five samples. This is shown in Table 11.

TABLE 11 : COMPARISON OF LAT VS. GRAM STAIN.

	G.stain +	**G.stain -**	**Total**
LAT positive	20	6	26
LAT negative	5	0	5
Total	25	6	31

Thus it was found that **both LAT and Gram staining helped at a presumptive diagnosis of pathogens in CSF. However both has their**

limitations in that LAT detected only the 5 specified organisms and Gram stain detected broad categories of organisms.

On comparing culture with Latex Agglutination test, it was noted that out of 26 LAT positives, culture yielded growth only in 13 samples. Of the 5 LAT negative samples, culture showed growth in all 5 samples. This is shown in Table 12.

TABLE 12-CORRELATION OF LAT AND CULTURE.

	Culture. +	**Culture. -**	**Total**
LAT positive	13	13	26
LAT negative	5	0	5
Total	18	13	31

Thus it was inferred that **Latex detected more number of fastidious organisms** from CSF samples when compared with culture. But **culture identified the other non fastidious organisms causing acute pyogenic meningitis where LAT had no role to detect them.**

The best method of detecting these organisms were evaluated separately and the results are given below. The organisms isolated in this study were S.pneumoniae, N.meningitidis, H.influenzae, Gr B Streptococci, E.coli K1, Klebsiella, and Staph.aureus.

Out of 19 S.pneumoniae detected, 15 were positive by gram staining, 5 were positive by direct culture, 9 by TI culture and 18 by latex agglutination test.

It was obvious that **LAT proved to be the best method for isolation of S.pneumoniae.**

Out of 3 meningococci detected, all 3 were detected by Gram staining, 2 were detected by TI culture and LAT. One was detected by direct culture. It was inferred that **Gram staining was proved to be an efficient method in detection of meningococci. Among the culture methods, TI culture was better than direct culture**.

Of the 3 H.influenzae detected, gram staining detected 2, direct culture none, TI culture identified one whereas LAT identified all the 3. Hence it was inferred that **LAT was proved to be the best method in the detection of H.influenzae**.

Of the one Gr B streptococci identified, all the 4 techniques identified that single isolate. Hence **for the detection of Gr B streptococci it was inferred that all 4 methods were equally efficient**.

Among the 2 E.coli K1 isolates detected, Gram staining identified one and direct, TI cultures, LAT identified both the isolates. **It was accepted that LAT and cultures were satisfactory for the identification of E.coli K1.**

Out of the 2 Klebsiella isolates detected, all 2 were detected by Gram staining, direct and TI cultures whereas LAT had no provision for detecting Klebsiella. And of the one S. aureus identified, Gram staining, direct and TI

cultures identified one isolate whereas LAT had no provision for detecting S. aureus. **It was inferred that the Gram staining, Direct culture and TI culture methods were valuable in the identification of Gram negative organisms as well as S.aureus where LAT has no role. This is shown below in Table 13.**

TABLE 13 DIFFERENT TECHNIQUES VS ORGANISMS DETECTED

Organisms	Gram staining	Direct culture	TI culture	LAT
S. pneumoniae(19)	**15(79%)**	**5(26%)**	**9(47%)**	**18(95%)**
N. meningitidis(3)	**3(100%)**	**1(33%)**	**2(67%)**	**2(67%)**
H.influenzae(3)	**2(67%)**	**0**	**1(33%)**	**3(100%)**
Group B Strep(1)	**1(100%)**	**1(100%)**	**1(100%)**	**1(100%)**
E. coli K 1(2)	**1(50%)**	**2(100%)**	**2(100%)**	**2(100%)**
Others(3)	**3(100%)**	**3(100%)**	**3(100%)**	**0**

It was clearly noted that LAT identified specified fastidious pathogens involved in Acute Bacterial Meningitis. Even though Gram staining also detected the organisms equally, the type of organism could not be ascertained by Gram staining. The culture methods quickly detected Gram negative organisms. Direct culture even though detected fastidious organisms, the number of isolates were minimum when compared with TI medium proving that TI culture is better for the isolation of fastidious organisms.

Of the three diagnostic tools utilized in this study for the etiological diagnosis of Acute Bacterial Meningitis, LAT was valuable in identification of the specific pathogens, while Gram stain could always resorted to for screening and presumptive identification of pathogens. Conventional culture methods help in confirmation of presumptive tests. The use of TI medium helps in isolation of the important fastidious pathogens.

DISCUSSION

DISCUSSION

Acute bacterial meningitis is a medical emergency, which warrants early diagnosis and aggressive therapy. Most often therapy for bacterial meningitis has to be initiated before the etiology is known.

Though the common pathogens associated with Community Acquired Acute Bacterial Meningitis are S.pneumoniae, H.influenzae and N.meningitidis, the etiological agents and their relative frequency may vary in different geographical areas. Despite the availability of potent newer antibiotics, the mortality rate remains significantly high in India and other developing countries, ranging from 16-32%.[5, 15, 31, 58] There is a need for a periodic review of bacterial meningitis worldwide, since the pathogens responsible for the infection vary with time, geography and patient age.

In this study, the prevalence of various pathogens in acute bacterial meningitis and the comparison of various methods of detection of pathogens in 200 suspected cases who were admitted in medical and paediatric wards in Government Rajaji Hospital, Madurai during the period between Feb 2008 to Jan 2009 were analysed.

Analysis of the patients in the present study showed that the overall incidence of Acute Bacterial Meningitis was 15.5% and it was noted that the incidence was more among males (61%) irrespective of age. The study by

Viswanath G et al[64] also revealed the incidence of Acute Bacterial Meningitis to be 19.8% and the incidence among males was 58%. Similar studies by **Mani R et al**[36] & **Surinder K et al**[55] also reported that the incidence of Acute Bacterial Meningitis in males was 71% and 61.5% respectively. These studies were in accordance with the present study.

The age group commonly involved in the present study in one year period was 0-6 years (36%). **Sameer Marji et al**[49] reported that 58% of acute bacterial meningitis was seen among the age group of 0-6years. Since his study was a retrospective study involving only children in four years period, there may be slight increase in the incidence in the above age group. **Mani R et al**[36] reported an incidence of only13.2% among the above age group. His study population mostly involved immunized children, hence a slight decrease in the incidence in this age group. Thus the age group involved in acute bacterial meningitis depends upon the study population and the period of study.

The common clinical finding in acute bacterial meningitis in this study was fever (100%). **Sameer Marji et al**[49] in their study also showed 92% of cases being presented with fever which is in support of this study. **Farag HFM et al**[19] also showed high fever as a constitutional symptom in 92.1% cases. It is well known that fever in meningitis is due to the inflammatory changes taking place in the meninges.

In this study, 6% of cases showed skin rash which was encountered especially in adults and it was found that most of the cases were due to N.meningitidis. **Sameer Marji et al[49]** reported an incidence of skin rash among 6% of their cases which is in accordance with the present study. The study by **Manchanda V et al[37]** showed 80% of their patients with meningococcal meningitis presented with skin rash. As his study was only on meningococcal meningitis, there was an increase in incidence. The skin rash is due to the endotoxemia produced by meningococcus causing hemorrhagic infarctions similar to Shwartzman reaction.

It was obvious in this study that the children involved in Acute Bacterial Meningitis markedly refused feeds (22%). **Farag HFM et al[19]** also showed that 28.6% of their children refused feeds. The refusal of feeds by children with acute bacterial meningitis may be due to the loss of appetite which may be secondary to other constitutional symptoms like fever and also by the action of toxins liberated by the microbes especially S.pneumoniae. In this study, most of the children presented with loss of appetite were proved to be affected by S.pneumoniae.

In this study, S.pneumoniae seemed to be the commonest (61%) pathogen among both adults and paediatric cases equally. Similar studies by **Mani R et al[36]** (61.8%), **Shameem et al[51] (44.7%), Sameer Marji et al[49](41%), Marlene L et al[39](37%), AI Khorasani et al[2](30.1%)** also showed S.pneumoniae to be the predominant pathogen causing acute bacterial meningitis. Similarly H.influenzae and Group B Streptococcus were commonly seen in paediatric age group and

N.meningitidis seen only in adults. **Tomas F.Zimba et al[59], Nihar Dash et al[41] and Vadher et al[60]** confirmed these findings. As Pneumococci are normal inhabitants of the human upper respiratory tract, this would have become a pathogen due to changes in factors like malnutrition, debilitation, alcoholism which were common in this study population and also the absence of anti-capsular antibodies which are common in extremes of age. The occurrence of H.influenzae among the children <6 yrs of age, may be due to the inadequate or undetectable levels of protective, anti PRP bactericidal antibodies at this age group. The occurrence of Group B Streptococcus in neonates might be due to the possibility of vertical transmission as the vaginal colonization of these organisms is common. Since meningococcal carriers were common among adults, the occurrence of meningococcal meningitis among adults as opportunistic infections is likely to occur. Moreover, as H.influenzae vaccine is commonly employed in the study population in the younger age group, S.pneumoniae might have predominated as the common pathogen.

It was noted in this study that 25 out of 31 positives (81%) showed the presence of organisms by Gram staining. **Viswanath et al[64]** showed the sensitivity of Gram staining to be 89%. Similar studies by **Rao B N et al[45]** and **Sherry et al[53]** showed 86% and 88% sensitivity respectively. All these studies correlated with the present study. Hence Gram staining can be taken as a highly reliable screening methodology for the diagnosis of Acute Bacterial Meningitis. Even though Gram staining detects the presence of organisms, the specific identification of an

organism cannot be done by Gram staining. Some other methodology may be needed for the identification and confirmation of these organisms. Thus Gram staining can be used only as a screening method and not as a confirmatory methodology.

Direct culture in this study isolated 39% of the organisms**. Mani R et al**[36] in their study reported 40.8% positivity, **Johny Vincent et al**[30] reported 43% culture positivity. All these studies correlated with this study. Similar studies by **Lidia Hristeva et al**[35] showed 81%, **Rao BN et al**[45] 86%**, Gaitonde et al**[24] 68% **and Hassib Narachi et al**[26] reported 80% sensitivity for direct culture. These studies did not correlate with the present study. It is well known that the CSF for culture if collected after the administration of antibiotics will show only low positivity. In all the latter studies, the CSF samples were collected prior to antibiotic therapy, hence increase in the culture yield. Whereas in this present study, most of the samples were collected after the administration of antibiotics and thus the yield of organisms were less.

However the cultures collected in TI medium yielded growth of all the fastidious pathogens like S.pneumoniae, N. meningitidis, H. influenzae, Gr B Streptococcus in a better way (58%). This may be due to the ingredients like Charcoal & Starch in TI medium which act as neutralizing and detoxifying agent and also the antimicrobial inhibitors like VCN Inhibitor, which is a main ingredient in this new medium. This medium protected the organisms from destruction even when slight changes in the pH of the medium which would

otherwise be toxic to the fastidious pathogens. Also this medium protected N.meningitidis at temp as high as 42 C and as low as 4 C. Thus culture in TI medium proved to be the best methodology of isolation of fastidious pathogens even after the administration of antibiotics.

In this study, LAT detected bacterial antigens in 84% of positive samples of acute bacterial meningitis. And the etiological agents were N meningitidis (8%), S.pneumoniae (69%), H.influenzae (11%), Gr B Streptococcus (4%), E.coli K1(8%). Interestingly 13 culture negative specimens were positive with LAT of which 9 were positive for S.pneumoniae, 2 for H.influenzae, 1 each for N.meningitidis and E.coli K1. Previous studies by **Manchanda V et al[37], Cuevax et al[17], Bhisitkhul DH et al[10]** had advocated the usefulness of bacterial antigen detection by LAT for the diagnosis of Acute Bacterial Meningitis esp. in situations like prior antibiotic administration. The results of the present study demonstrated the same. Hence LAT is a useful method in the diagnosis of Acute Bacterial Meningitis irrespective of antimicrobial therapy. But LAT could detect only the 5 specified organisms, which are considered to be more specific and rarely isolated meningitis pathogens. However it is rapid enough and an adjunct laboratory test for diagnosing bacterial meningitis particularly in pretreated cases.

SUMMARY

SUMMARY

The study on the Prevalence and diagnosis of Acute Bacterial Meningitis in a tertiary care centre revealed the incidence of Acute Bacterial Meningitis was 15.5% and among them 61% cases were males, 36% of the affected were in the age group of 0-6 yrs. 100% of the cases presented with fever, 6% of the cases with skin rash and 22% of the paediatric cases refused feeds. S.pneumoniae was proved to be the commonest pathogen among both the adult and paediatric populations.

Among the 31 lab proven positives, Gram staining showed the presence of organisms in 81% of positives. As the organisms were not confirmed by Gram staining, it was considered as a screening method in the diagnosis of Acute Bacterial Meningitis. Direct culture yielded growth in 39%, TI culture in 58% of positives. All the fastidious pathogens like S.pneumoniae, N.meningitidis, H.influenzae, Gr B Streptococci were isolated in the TI medium revealing that TI medium is better than direct culture in the isolation of fastidious organisms. Latex agglutination test used in this study detected bacterial antigens in 84% of positive cases and the etiological agents were S.pneumoniae, N.meningitidis, H.influenzae, Gr B Streptococci, E.coli K1. 13 culture negative specimens were positive by LAT. As LAT has no influence over the antimicrobial therapy, it detected organisms readily. It is a rapid method and can be used in the pretreated cases.

Organism wise detection revealed that S.pneumoniae, was readily detected by Latex agglutination test. N.meningitidis was readily detected by Gramstaining, H.influenzae readily by LAT, Gr B Streptococcus was detected equally by all the techniques. E.coli K 1 was detected in cultures and LAT equally whereas the other Gram negatives were detected by Gram staining and culture equally. Thus LAT proved to be the rapid detection test for 5 specified organisms whereas Gram staining and culture detected the organisms less readily. Among the cultures, culture in TI medium isolated more fastidious pathogens. Hence Gram staining can be used as a screening test and cultures as a confirmatory test. Since culture in TI medium is a laborious procedure, it can be reserved for the isolation of fastidious pathogens whereas Direct culture for all isolations. Latex agglutination test can be used as a confirmatory for the specified organisms. Even though it is rapid, LAT is costly. Hence can be used in referral laboratories.

CONCLUSION

CONCLUSION

The study on the Prevalence and diagnosis of Acute Bacterial meningitis in a tertiary care centre revealed the following findings

1) Among the suspected acute bacterial meningitis cases, 15.5% were proven positive using various techniques.

2) Among the positive cases, 61% were males.

3) The common age group involved was 0-6 years irrespective of sex.

4) S.pneumoniae, N.meningitidis, H.influenzae, Gr B.Streptococcus and E.coli K1 were the common organisms detected as causative agents. S.pneumoniae was the commonest organism isolated in all age groups. N.meningitidis was more common in adults and H.influenzae and Gr B.Streptococcus was more common in paediatric age group.

5) 81% of the cases were detected by Gram staining, 39% by direct culture, 58% by TI culture and 84% by Latex agglutination test.

6) Since, Gram staining cannot detect specified organisms it can be used as a screening methodology. Even though cultures are the gold standard for confirmation, Direct culture does not yield growth of fastidious pathogens readily whereas cultures in TI medium yielded growth of fastidious organisms readily revealing TI medium as the best method for culture. But it a laborious

method for the preparation of media and the reagents are not readily available in India. Hence Direct culture has to be relied for the confirmation and TI culture for the confirmation of fastidious pathogens.

7) Latex Agglutination Test seems to be the more rapid and specific method for identification of 5 specified pathogens like S.pneumoniae, N.meningitidis, H.influenzae, Gr B.Streptococcus and E.coli K .The prohibitive cost of LAT restricts the use of this test, except in reference laboratories.

OUTCOME OF STUDY

OUTCOME OF STUDY

This study clearly proved that acute bacterial meningitis is still a problem among both adults and paediatric cases in the study population. Early diagnosis and treatment is very much needed in this emergency. For the early diagnosis, rapid, easily available, less laborious and cost effective methods are needed in this population. Of the four techniques evaluated in this study, Gram staining can be the only method which satisfies the above needs but it will not help in identifying the pathogens upto the species level. Hence for all the suspected cases, Gram staining can be suggested as a screening technique and the treatment can be initiated accordingly. For the confirmation of suspected pathogens, cultures can be adopted. For the final diagnosis of specified pathogens commonly responsible for meningitis, rapid tests like LAT can be recommended but with scrutinization as the kits are very costly.

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68. Wilder Smith E, Chow KM et al. Group B Streptococcal meningitis in adults : Recent increase in south east Asia . Aust N Z J Med2000; 30:462-5.

69. William JM. Rapid and reliable techniques for the laboratory detection of bacterial meningitis. Am J Med 1983; 119-23.

ANNEXURE-1

PREPARATION OF GRAM'S STAIN REAGENTS

1.Methyl violet – Primary stain

Methyl violet 10g

95% ethyl alcohol 100ml

Distilled water 1 L

2.Gram's Iodine – Mordant

Iodine 10g

Potassium Iodide 20g

Dist. Water 1 L

3. Acetone – Decolouriser

4. Dil. Carbol Fuchsin – Counter stain

Basic fuchsin 0.3 g

95% Ethyl alcohol 10ml

Phenol crystals, melted 5ml

Dist. Water 95ml

Dissolve fuchsin in alcohol. Add the 5% phenol solution. Let stand overnight. Filter through coarse filter paper.

ANNEXURE – 2 PREPARATION OF MEDIA

PREPARATION OF BLOOD AGAR PLATE: TSA + 5% Sheep Blood

To prepare 500ml of molten agar in 1litre flask,

1. Add 20g of agar into 500ml of water. Heat to dissolve.
2. Autoclave at 121C for 20 mins. Cool to 50C.
3. Add 5% sterile, defibrinated sheep blood (25 ml sheep blood is added to 500 ml of molten agar).
4. Dispense 15-20ml in to Petri dishes. To remove the bubbles media can be flamed. Allow to solidify, dry out. Place the plates in a plastic bag and store at 4C.

PREPARATION OF CHOCOLATE AGAR PLATE:

To prepare 500ml of molten agar in 1litre flask,

1. Add 20g of trypticase soy agar in to 500 ml of water.Heat to dissolve.
2. Autoclave at 121C for 20 mins. Cool to 50C.
3. Add 5% sterile, defibrinated sheep blood (25 ml sheep blood is added to 500 ml of molten agar).Then place in a hot water bath at no more than 80C for 15 mins. or chocolate colour is achieved. Then to 50C.
4. Dispense 15-20ml in to Petri dishes. Allow to solidify, dry out. Place the plates in a plastic bag and store at 4C.

PREPARATION OF CHOCOLATE AGAR PLATE WITH ISOVITALEX:

To prepare 500ml of molten agar in 1litre flask,

1. Add 20g of trypticase soy agar in to 500 ml of water.Heat to dissolve.
2. Autoclave at 121C for 20 mins. Cool to 50C.
3. Add 5% sterile, defibrinated sheep blood (25 ml sheep blood is added to 500 ml of molten agar).Then place in a hot water bath at no more than 80C for 15 mins. or chocolate color is achieved. Then to 50C.
4. Add the growth supplement (IsoVitaleX) to a final concentration of 1%. Mix the ingredients by swirling the flask; avoid forming bubbles.
5. Dispense 15-20ml in to Petri dishes. Allow to solidify, dry out. Place the plates in a plastic bag and store at 4C.

PREPARATION OF TRANS-ISOLATE MEDIUM:

1. Use glass serum bottles with flange-type, slotted rubber plugs and aluminium crimp seals. Any size from 20 or 30 ml capacity or greater is appropriate, provided that the combined volume of solid and liquid phases equals approximately one-half of the capacity of the bottle.

Diluent for solid liquid phases:

3-(N-Morpholino propane sulphonic acid) MOPS buffer 0.1 M,pH7.2 -20.93g

Distilled water up to 1000ml after adjusting to pH 7.2 with NaOH.

2. SOLID PHASE:

Activated Charcoal 2.0g

Soluble Starch 2.5g

Agar 10.0g

Suspend in 500ml of MOPS buffer and add a magnetic bar to the flask. Heat on a magnetic stirrer-heater to dissolve the starch and the agar. While stirring to keep the charcoal in suspension, dispense 5ml to 20ml serum bottle. Cap each bottle with a piece of aluminium foil and autoclave in metal baskets at 121C for 20 mins. Remove from the autoclave and slant the baskets until the bottles cool, so that the apex of the agar reaches the shoulder of each bottle.

3. LIQUID PHASE:

Tryptic soy broth 30g

Gelatin 10g

MOPS buffer 500ml

Heat the medium to dissolve the gelatin and avoid coagulation. Then autoclave at 121C for 15 mins. Dissolve by mixing vigorously in 5ml of MOPS buffer. Pass through a 0.22micron membrane filter to sterilize before adding to the medium.

4. Dispense 5ml of broth aseptically in to each of the bottles containing the solid-phase slants. Seal with sterile rubber stoppers and aluminium caps. Use a hand-crimping tool to fasten the aluminium caps if an automated system is not available.

T-I bottles can be stored for atleast 2 years if tightly capped and stored at 4C. In the refridgerator, the liquid phase becomes gelatinous but re-liquifies at room temperature. Before inoculation, the bottles should be pre-warmed in the incubator or allowed to reach room temperature.

ANNEXURE-3

Case Investigation Form

Date:

Patient's Name: Patient's I.D. No.:

Age / Sex:

Parents / Spouse Name:

Address:

Date of onset of illness:

Date of hospitalization:

Occupation:

Clinical signs & symptoms (with duration)

Immunization History:

Treatment History:

Results of previous investigations (if any):

Any other relevant information:

Specimen details:

Nature of specimen (s)	Date of collection	Investigation required

Details of sender:

Signature:

Name of sender: Telephone no:

ANNEXURE-4

LABORATORY FORM

Date:

Patient's Name: Patient's I.D. No.:

Age / Sex: Laboratory Reference No.:

Specimen details:

Type of Specimen	Date of Collection	Date of Receipt in lab	Type of test	Remarks (if any)	Result

Interpretation:

Details of Investigator:

Name:

Signature

Address:

FIGURE 1

GRAM STAIN SHOWING GRAM POSITIVE COCCI IN DIPLOS

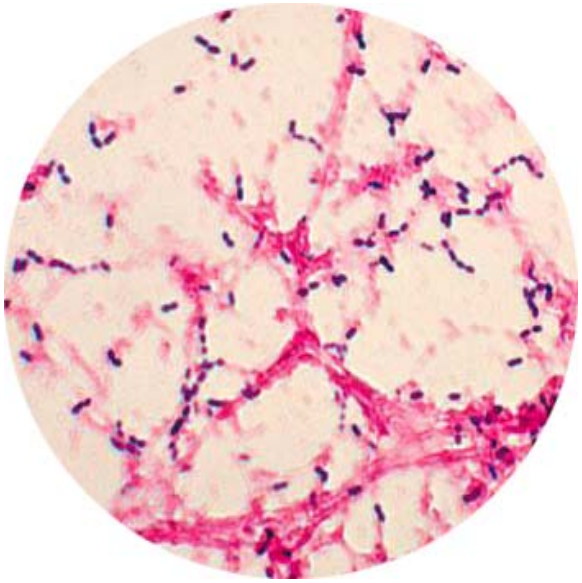


FIGURE 2

TRANS- ISOLATE MEDIUM

FIGURE 3

BLOOD AGAR PLATE WITH N.MENINGITIDIS COLONIES

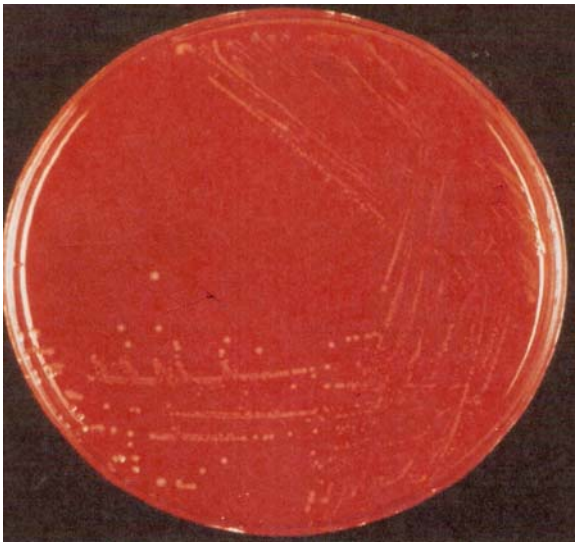


FIGURE 4

BAP SHOWING ALPHA HEMOLYTIC COLONIES

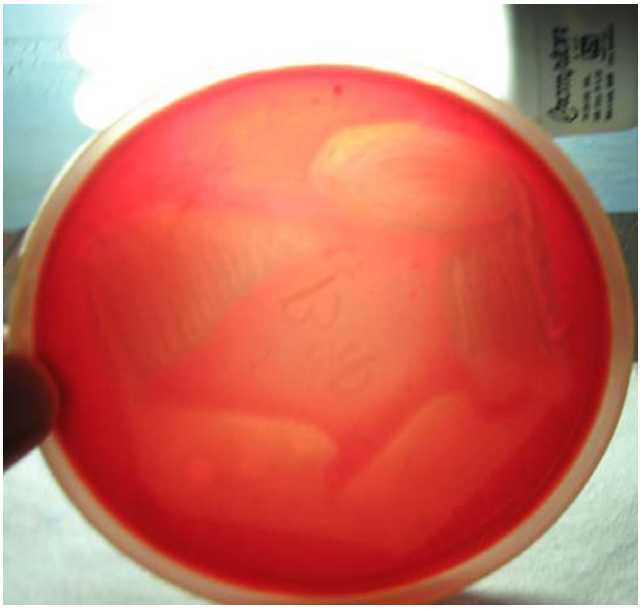


FIGURE 5
OPTOCHIN SENSITIVITY TEST

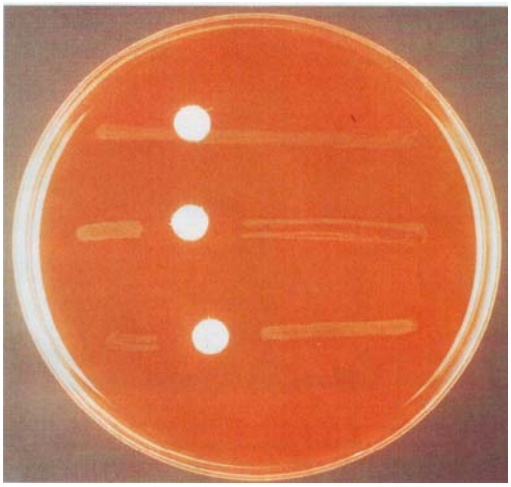
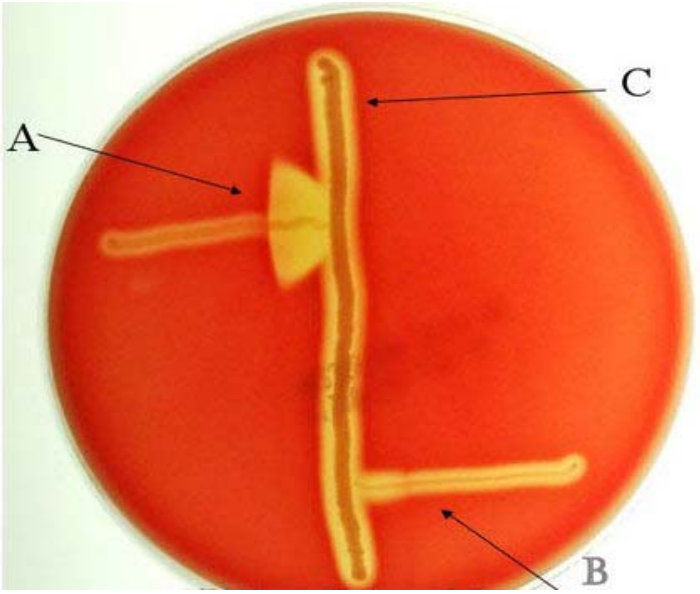


FIGURE 6
CAMP TEST



A – Gr B Streptococci B- Gr A Streptococci C – Staph.aureus

LATEX AGGLUTINATION KIT

FIGURE 6

FIGURE 7

FIGURE 9

LAT POSITIVE FOR N.MENINGITIDIS ACYW135

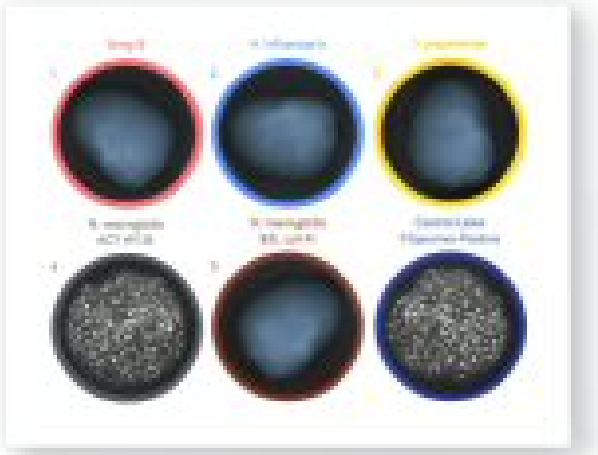


FIGURE 10

BILE SOLUBILITY TEST

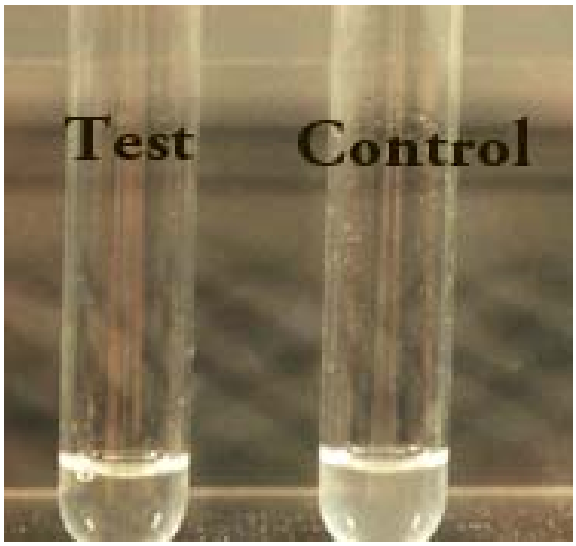


FIGURE 1 AGE WISE DISTRIBUTION

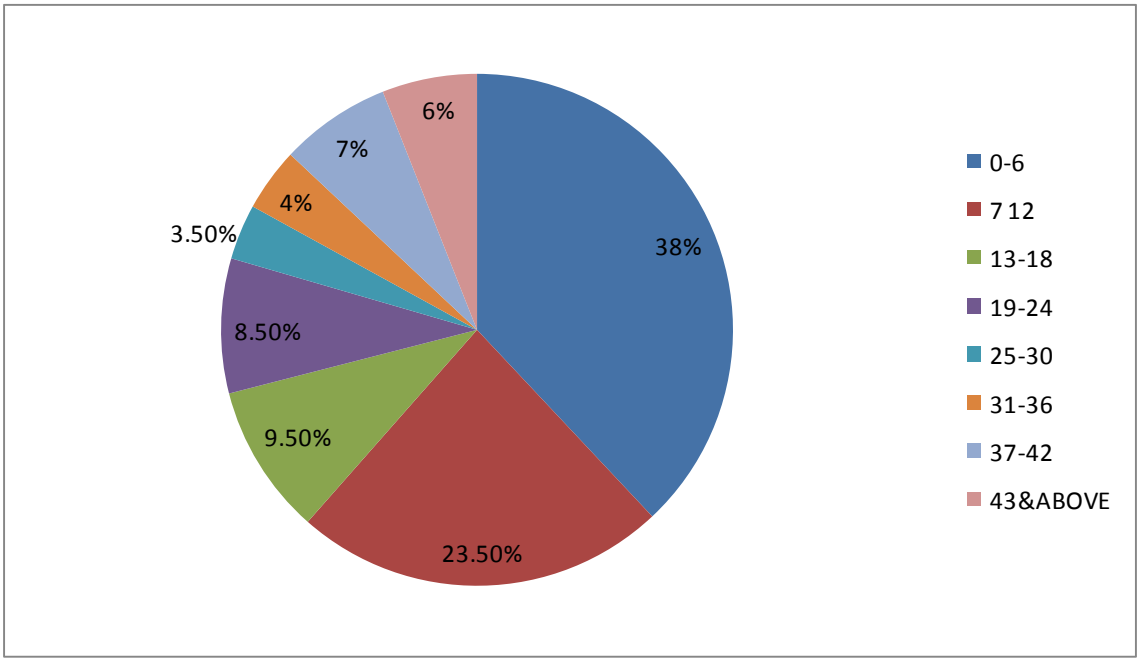


FIGURE 2 SEX WISE DISTRIBUTION

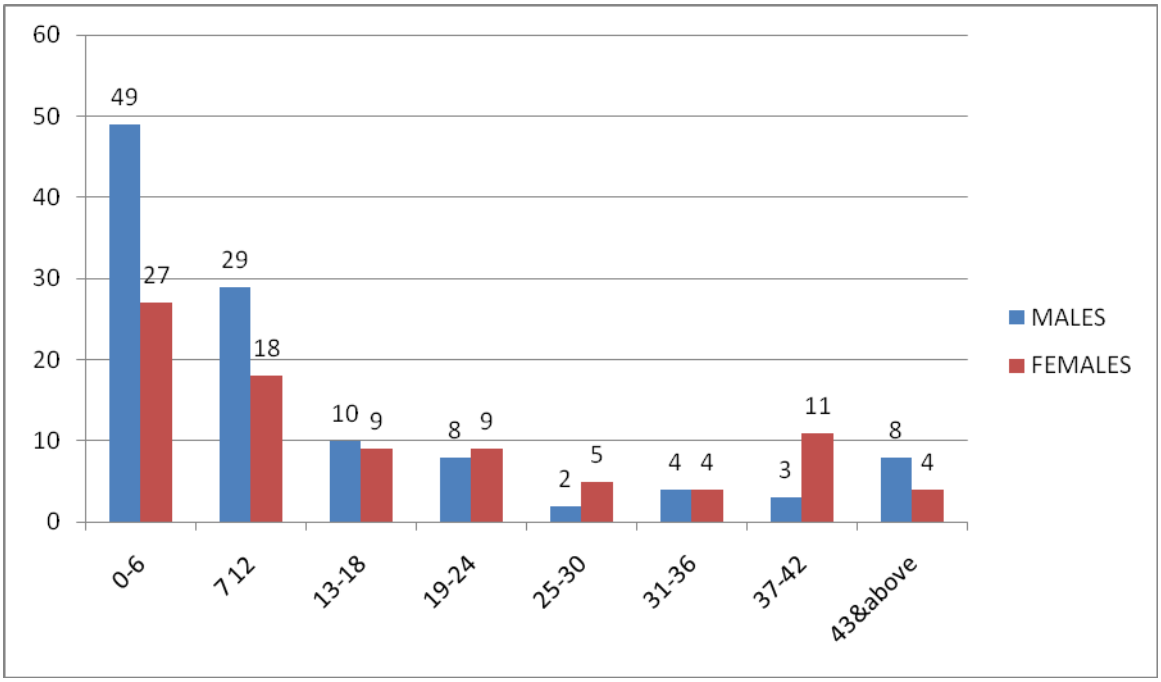


FIGURE 3-TOTAL INCIDENCE VS CLINICAL FEATURES

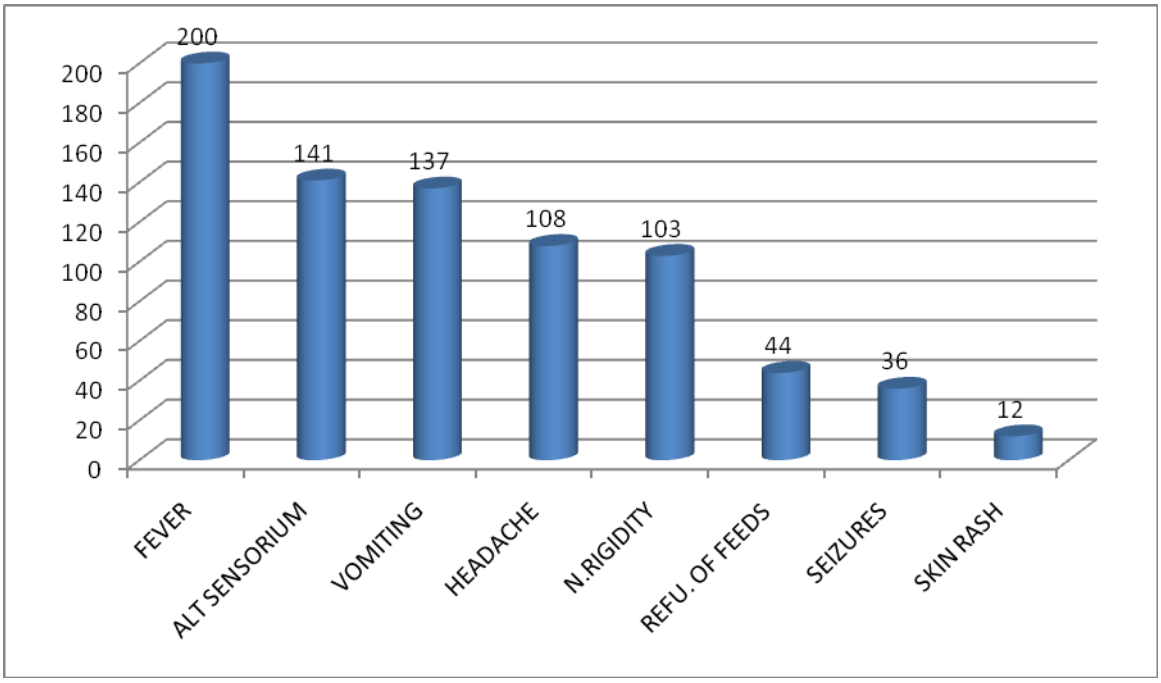


FIGURE 4 INCIDENCE OF ADULTS AND PAEDIATRICS AS PER CLINICAL CONDITIONS

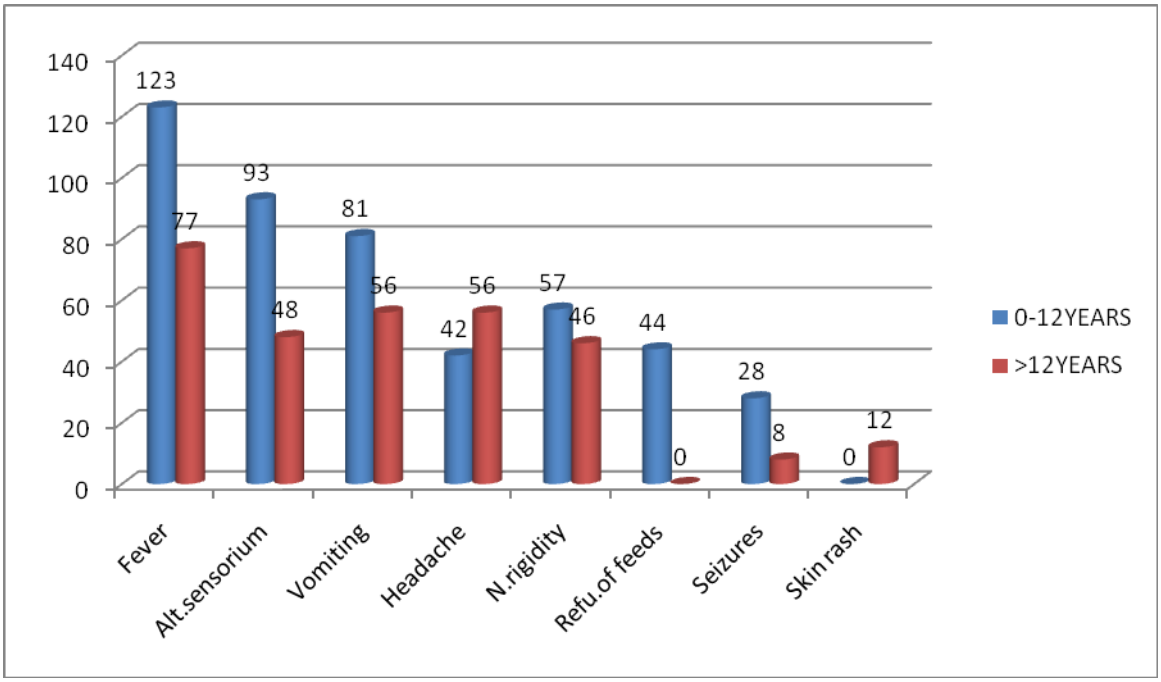


FIGURE 5 ORGANISMS ISOLATED

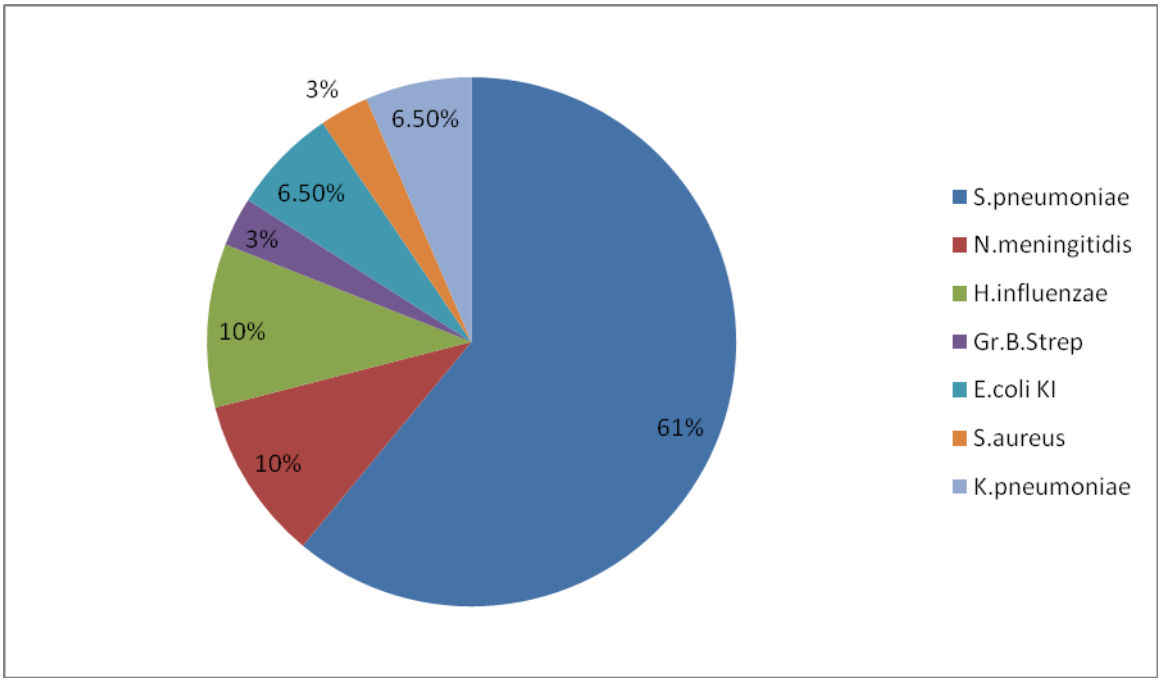
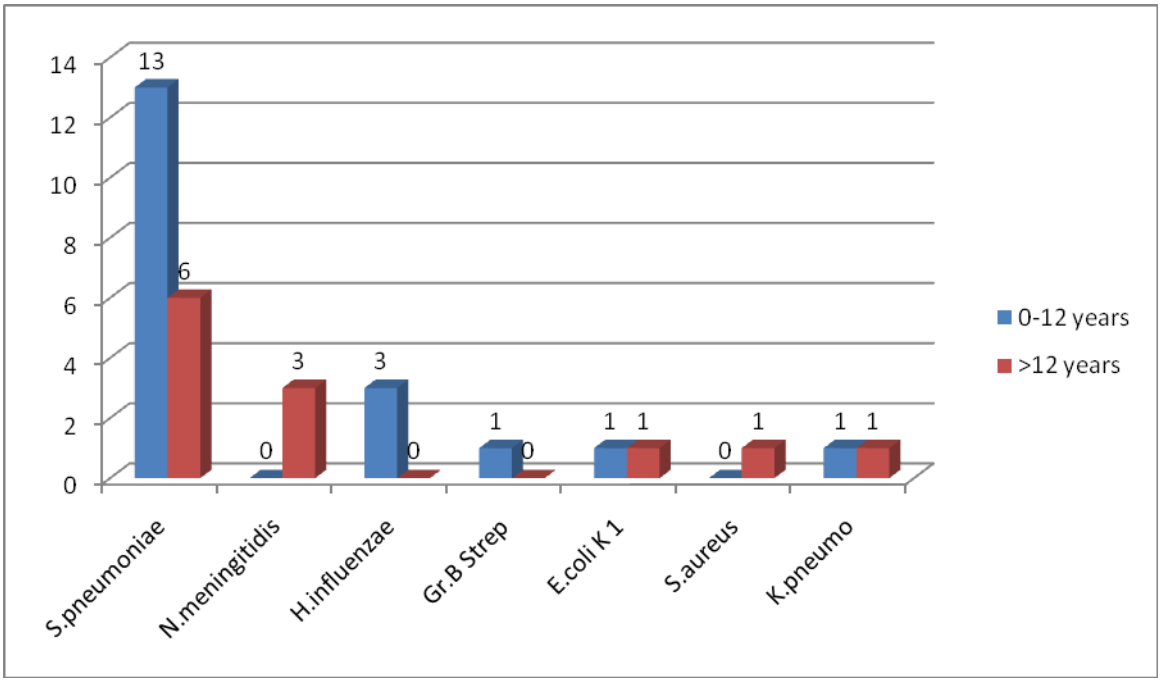


FIGURE 6 ORGANISMS ISOLATED AMONG ADULT AND PAEDIATRIC AGEGROUP



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K.Dis.No.4735/E4/1/2008

Government Rajaji Hospital,
Madurai – 625 020.

Date : 11.06.2008

19/6/2008

Sub: Establishment – Government Rajaji Hospital, Madurai – Ethical Committee Projects approved by the Committee Intimation – Sent- Regarding.

% % % % %

The Ethical Committee of the Government Rajaji Hospital, Madurai was held at 12.30 Noon on 05.06.2008 at the Dean's Chember Government Rajaji Hospital, Madurai and the following Projects were approved unanimously by Committee Members

S.No	Name of the PG	Course	Name of the Project Approved
01	Dr.N.Anuradha	Microbiology	Bacteriological profile of Acute pyogenic meningitis.
02	Dr.J.Preeth	Microbiology	Evaluation of various samples for Microbiological Diagnosis of Ventilator associated Pneumonia

Please note that the investigator should adhere the following :

1. She / He should get a detailed informed consent from the patients / participants and maintain Confidentially.
2. She / He should carry out the work without detrimental to regular activities as Well as without extra expenditure to the institution to Government.
3. She / He should inform the Institution Ethical Committee in case of any change Of study procedure site and investigation or guide
4. She / He should not deviate for the area of the work for which applied for Ethical clearance
5. She/He should inform the IEC immediately, in case of any adverse events or Serious adverse reactions.
6. She / He should abide to the rules and regulations of the Institution.
7. She / He should complete the work within the specific period and apply for if any Extension of time is required She should apply for permission again and do the work.
8. She / He should submit the summay of the work to the Ethical Committee on Completion of the wor
9. She / He should not claim any funds from the Institution while doing the Work or on completion.
10. She / He should understand that the members of IEC have the right to monitor the Work with prior Intimation.

Transmitted
19/6/08

11/6/08

DEAN/ CHAIRMAN,
ETHICAL COMMITTEE,
GOVERNMENT RAJAJI HOSPITAL,
MADURAI.

To
The above Post Graduates though the Head of the Departments Concerned.